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Effect of Linoleic Acid and Hydraulic Retention Time on Anaerobic Sulfate Reduction in
High Rate Reactors

by

Purnima Mallelwar

A Thesis

Submitted to the Faculty of Graduate Studies
through the Department of Civil and Environmental Engineering
in Partial Fulfillment of the Requirements for
the Degree of Master of Applied Science at the
University of Windsor

Windsor, Ontario, Canada

2013

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Effect of Linoleic Acid and Hydraulic Retention Time on Anaerobic Sulfate Reduction
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ABSTRACT

Metal precipitation using sulfide produced biologically by sulfate reducing bacteria (SRB) is an attractive alternative for the treatment acid mine drainage (AMD). The process can be affected by competition from methane producing bacteria (MPB) when organic carbon is limited. This study shows that linoleic acid (LA) can be used to selectively inhibit MPB in high rate semi-continuous upflow anaerobic hybrid reactors (UAHR) to make more organic carbon available to SRB. At a slug LA dose of 1000 mg/L in LA-treated UAHR, ~ 100% of organic carbon reduced was diverted to sulfate reduction as compared to 74 – 59% in the control UAHR at hydraulic retention time (HRT) varying between 50 – 7 days. Sulfate reduction of 99 - 85% and sulfide levels of 470 – 500 mg/L were maintained in LA-treated UAHR as compared to sulfate reduction of 94 - 58% and sulfide levels of 450 – 280 mg/L in the control UAHR.

DEDICATION

To my parents

ACKNOWLEDGEMENTS

It is with immense gratitude that I acknowledge the support, guidance, and availability of my advisor, Dr. Rajesh Seth throughout my research and completion of my thesis. I am very thankful to my committee members, Dr. Chris Weisener and Dr. Nihar Biswas for taking their time to review my thesis and provide valuable comments.

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LIST OF ABBREVIATIONS

AMD	acid mine drainage
AMPB	acetoclastic methane producing bacteria
ASRB	acetoclastic sulfate reducing bacteria
BOD	biological oxygen demand
COD	chemical oxygen demand
CSTR	continuous stirred tank reactor
HMPB	hydrogenotrophic methane producing bacteria
HRT	hydraulic retention time
HSRB	hydrogenotrophic sulfate reducing bacteria
IC	inorganic carbon
LA	linoleic acid
LCFA	long chain fatty acid
MPB	methane producing bacteria
OA	oleic acid
SA	stearic acid
SAMD	simulated acid mine drainage
SL	sulfidogenic liquor
SRB	sulfate reducing bacteria
TOC	total organic carbon
TS	total sulfide
UAHR	upflow anaerobic hybrid reactor
UASB	upflow anaerobic sludge blanket
VFA	volatile fatty acid

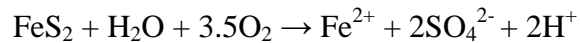
CHAPTER I

INTRODUCTION

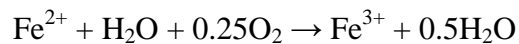
1.1 Background

Management of acid mine drainage (AMD) is one of the biggest challenges faced by the mining industry. As the name suggests, AMD is the outflow of acidic water from abandoned mines and the mine tailing ponds. It is characterized by low pH and high dissolved metal concentrations. AMD can contaminate surface waters or seep into groundwater, causing major environmental problems (Johnson et al., 2002). AMD is generated by biological and chemical oxidation of sulfide ores, especially pyrite (FeS_2). The oxidation of pyrite is summarised in the following equations (Blodau, 2006).

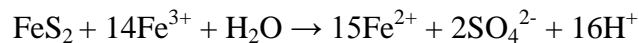
Pyrite oxidation by O_2 :



Ferrous ion oxidation by O_2 :



Pyrite oxidation by ferric ion:



The various modes of AMD treatment can be categorized as chemical and biological methods. Chemical methods such as hydroxide and carbonate precipitation have certain disadvantages. For example, chemical precipitation using lime is simple and cost effective but it produces large volumes of bulky and hazardous waste. It needs continuous maintenance and may not meet demanding upcoming environmental standards of waste

disposal and metal removal (Wilson 1984). Chemical precipitation using sulfide is more attractive in terms of efficiency of metal removal and compactness of sludge formed. However, difficulty in transport and handling, and cost of reagent (NaS, CaS) makes it unattractive.

Over the last few decades biological treatment (biological sulfate reduction) has become popular because of the low cost and low energy requirement of the anaerobic digestion process (Kaksonen and Puhakka, 2003). This process employs a special class of microbes termed sulfate reducing bacteria (SRB). SRB use organic carbon and sulfate for their growth and in doing so reduce sulfate to sulfide. This biologically produced sulfide thus can be used for metal precipitation giving the two-fold benefit of sulfate and metal removal from AMD. A two-stage process option has been discussed in literature whereby the sulfide is produced in stage 1 through biological sulfate reduction and then used in stage 2 of chemical precipitation for metal removal from AMD. Even though this treatment looks attractive, co-existence of the methane-producing bacteria (MPB) competing for the organic carbon source is a major challenge associated with the process. This competition plays an important role when organic carbon content is limiting as a part of the carbon source is not available for sulfate reduction, making the process less efficient (Weijma et al., 2000). MPB inhibition is been studied when hydrogen as an end product was desired. Heat treatment was found effective but it is economically unattractive in full scale applications (Duangmanee et al., 2007). Use of chemical inhibitor 2-bromoethane sulfonate is not popular because of high cost and problem of discharge to environment. Another group of chemicals long chain fatty acids (LCFAs) have been shown to be inhibitory to MPB (Angelidaki & Ahring, 1992; Hanaki et al., 1981; Kim et al., 2004;

Koster & Cramer, 1987). LCFAs are an attractive alternative because of its cost effectiveness and easy availability. More recently, Sharma and Biswas (2010) have shown in batch reactors that linoleic acid (LA), an LCFA with 18 carbon atoms and 2 double bonds, can be used in biological sulfate reduction to selectively inhibit MPB without affecting the activity of SRB. Biswas (2012) demonstrated the same in suspended growth semi-continuous stirred tank reactors. However, the potential of LCFA to selectively inhibit MPB so that SRB can function efficiently at a low substrate concentration has not been studied in high rate reactors. The possibility of same being true is tested in this study.

1.2 Objective

The main objective of this thesis was to investigate the effect of linoleic acid and hydraulic retention time on biological sulfide production in anaerobic high rate reactors as stage 1 of a two-stage process. A further objective was to examine the use of sulfide produced in stage 1 for metal precipitation from AMD in stage 2.

1.3 Scope

The scope of this thesis research was to:

- Design, build, start-up and operate two upflow anaerobic hybrid reactors (UAHR), as a stage 1 of the two stage process to produce sulfide by biological sulfate reduction;
- Investigate the effect of linoleic acid (LA) on process performance of one UAHR reactor fed semi-continuously at 50 day hydraulic retention time (HRT) and compare it with the control UAHR with no LA addition;

- Investigate the effect of varying hydraulic retention times on the difference in process performances of the LA treated UAHR and the control UAHR;
- Conduct batch studies to evaluate the efficiency of sulfide produced in UAHR reactors in stage 1 to precipitate copper from simulated AMD in stage 2 of the two stage process

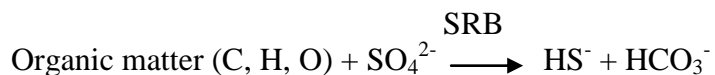
CHAPTER II

REVIEW OF LITERATURE

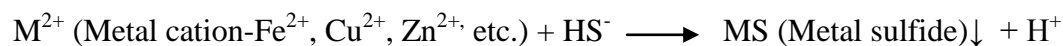
2.1 Overview

Anaerobic biological treatment of acid mine drainage is a promising alternative to conventional treatment processes such as neutralization and chemical precipitation (Kaksonen & Puhakka, 2007; Luptakova et al., 2007). Low cost, better sludge thickening characteristics and the possibility of recovery of metals are some advantages of metal precipitation using biologically produced sulfide over the conventional treatments. The biological treatment involves two steps.

1. Biological sulfide production using an anaerobic digestion process



2. Metal precipitation using the sulfide produced in the first step



These two steps can be carried out in a single stage process or a two-stage process. In a single stage process, biological sulfate reduction to sulfide and the metal precipitation is carried out in one reactor, which may expose the anaerobic consortium to low pH and toxic metal concentration. This can be avoided with the two-stage process as the sulfate reduction and the metal sulfide precipitation is carried out in separate reactors. A schematic diagram of the two-stage process is shown in Figure 2.1.

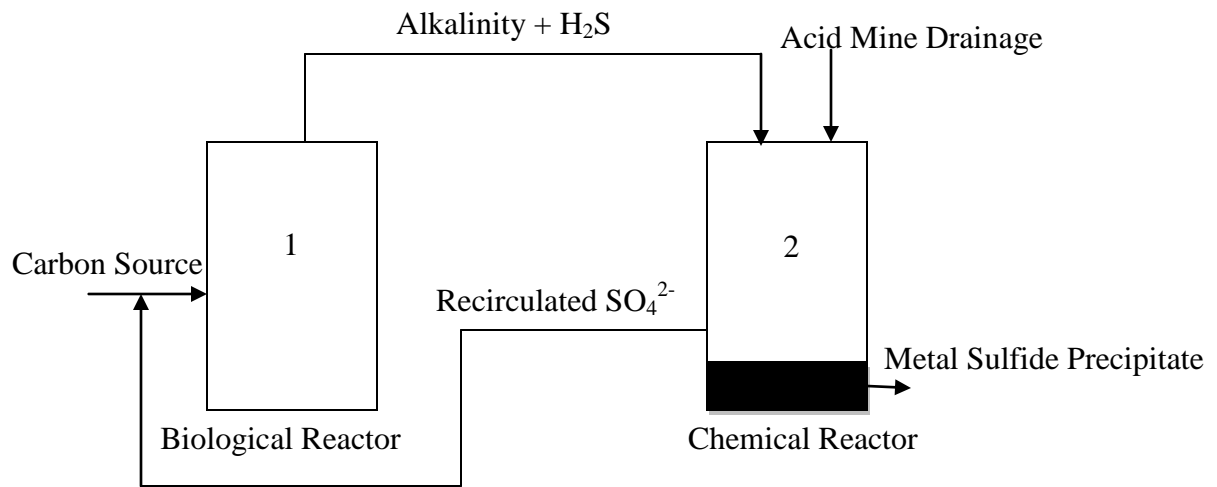


Figure 2.1 Two-stage process for treatment of AMD (adapted from Al-Ani et al., 1995)

An important factor on which metal precipitation in the second stage depends is sulfide production in stage 1. The major challenge associated with stage 1 is the competition of sulfate reducing bacteria (SRB) with methane producing bacteria (MPB) for the available substrate. The basics of anaerobic digestion process and the factors affecting the competition and inhibition of MPB will be discussed in this chapter.

2.2 Anaerobic Digestion

The anaerobic digestion process is one of the frequently used method to stabilize the wastewater containing dissolved and suspended organic matter (Toerien & Hattingh, 1969). It has become popular in many full-scale applications because of its cost effectiveness and energy efficiency (Lettinga, 1995). The digestion process can be divided into four major steps: hydrolysis, acetogenesis, acidogenesis, methanogenesis and/or

sulfidogenesis (Figure 2.2). Methanogenesis and sulfidogenesis are the terminal steps in the anaerobic digestion process.

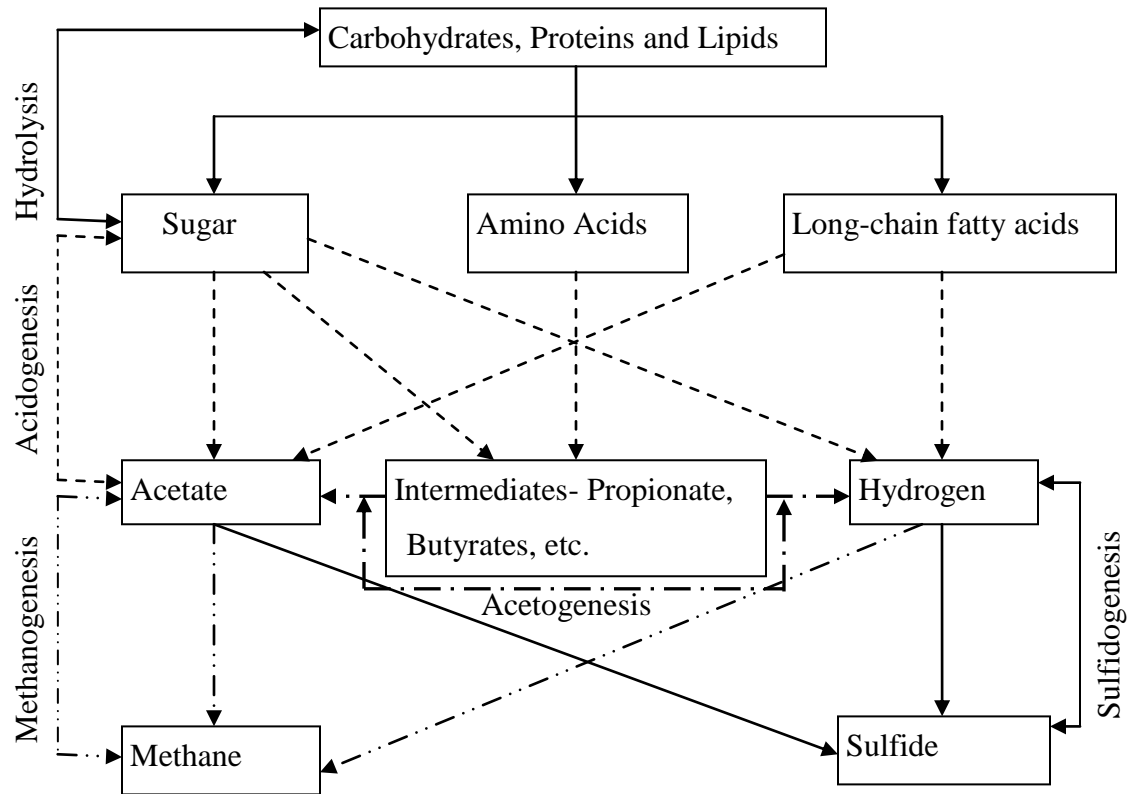
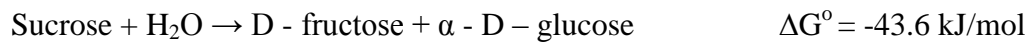
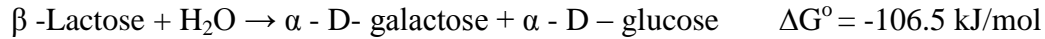


Figure 2.2 Pathways of competition between MPB and SRB during anaerobic digestion of organic matter. [Adapted from Visser et al., 1996]

2.2.1 Hydrolysis

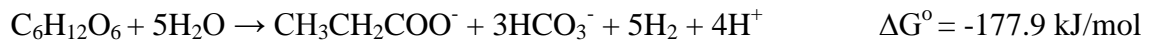
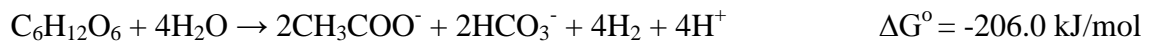
This is the first step in the anaerobic digestion process. Complex organic matter such as carbohydrates, fat and protein, is degraded into simpler monomers (monosaccharides, fatty acids, amino acids) by using extracellular enzymes secreted by

hydrolytic micro-organisms (Veeken & Hamelers, 1999). Hydrolysis is considered a rate-limiting step in the anaerobic digestion process (Bitton, 1994). Examples of hydrolytic reactions and their free energy changes (ΔG^0) at temperature 237°K and 1 atmospheric pressure are given below (Thauer et al., 1977).



2.2.2 Acidogenesis

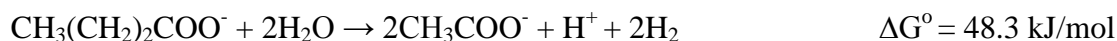
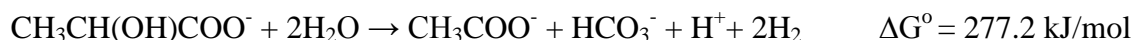
This is the second step in the anaerobic digestion process. The product of the hydrolysis step is further degraded into alcohols, organic acids, hydrogen, carbon dioxide and volatile fatty acids (VFA) such as acetic acid, propionic acid and butyric acid by fast growing fermentative acid forming bacteria (Boone, 1982). Examples of acidogenic reactions and their free energy changes (ΔG^0) at temperature 237°K and 1 atmospheric pressure are given below (Thauer et al. 1977).



2.2.3 Acetogenesis

The products of acidogenic step are converted into H_2 , CO_2 and acetic acid by acetogenic bacteria. This step is very sensitive to hydrogen partial pressure. Low hydrogen

partial pressure is needed to carry out the acetogenic reaction which is achieved with hydrogen scavenging micro-organism (Mata-Alvarez, 2002). The products of the acidogenic and acetogenic phases can be utilized by two different groups of bacteria namely methane producing bacteria (MPB) and sulfate reducing bacteria (SRB). Figure 2.2 shows acetate and hydrogen conversion pathways, which give different end products of anaerobic digestion. Examples of acetogenic reactions and their free energy changes (ΔG°) at temperature 237°K and 1 atmospheric pressure are given below (Thauer et al. 1977).



2.2.4 Methanogenesis

Methanogenesis is one of the terminal steps occurring in the anaerobic digestion process. It is carried out by a special group of bacteria called methane producing bacteria (MPB) which can be categorized as i) acetate utilizing- acetoclastic MPB and ii) hydrogen and CO_2 is utilizing- hydrogenotrophic MPB (Demirel & Scherer, 2008). About 70% of methane is formed by acetoclastic MPB and the remaining by hydrogenotrophic MPB (Conrad, 2007; Gujer & Zehnder, 1983). MPB are very slow growing organisms as compared to acid forming organisms. The doubling time of MPB is a few days whereas it is a few hours for acid formers (Bitton, 1994). Balance between the activity of MPB and the acid formers is very crucial, as excess of the later may cause an accumulation of volatile fatty acids and hydrogen, which is unhealthy for the digester operation.

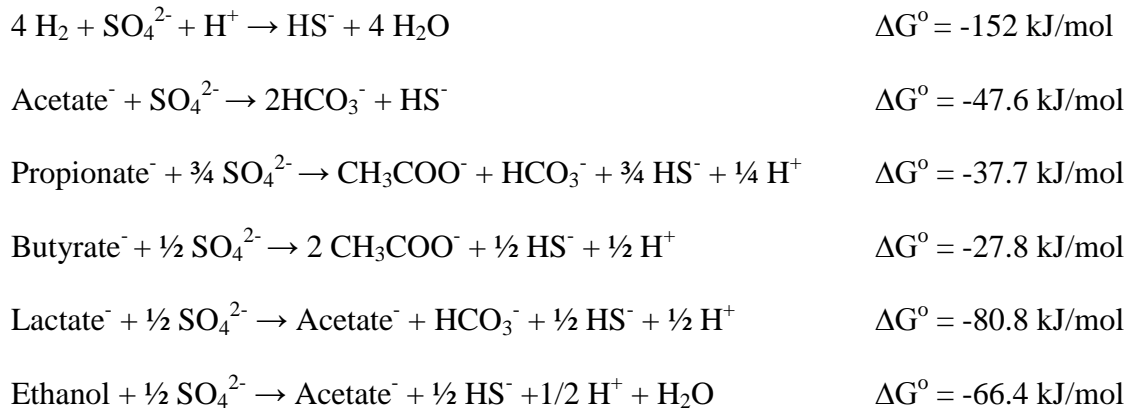
Methanogenic reactions and the standard free energy change of the reactions (ΔG°) at temperature 237°K and 1 atmospheric pressure is given below (Thauer et al., 1977).



2.2.5 Sulfidogenesis

Sulfidogenesis is another terminal step occurring during the anaerobic digestion process accomplished by SRB when sulfate is present in the wastewater. SRB use the organic carbon source as an electron donor and sulfate as an electron acceptor for their growth (Salmond & Whittenbury, 1985). In this process, they reduce sulfate to sulfide where a very small fraction of the sulfide produced is used for cell synthesis and the remaining sulfide is expelled or wasted. These are called assimilatory and dissimilatory sulfate reduction pathways respectively (Widdel, 1988).

SRB are very diverse in terms of their metabolic pathways, as they can use acetate as well as a variety of other compounds, such as organic acids, alcohols, VFA and at times sugars and long chain fatty acids, as their carbon source or electron donors (Hao et al., 1996; Oude Elferink et al., 1994; Weijma et al., 2000). Depending upon these criteria of substrate utilization, the SRB can be categorized as acetate utilizers and non-acetate utilizers (Madigan, 2000). Non-acetate utilizers oxidize the organic substrate such as organic acids, VFA, and alcohols, to acetate and acetate utilisers oxidize acetate to CO_2 . Some sulfate reducing reactions and their free energy change (ΔG°) at temperature 237°K and 1 atmospheric pressure are presented below (Thauer et al., 1977).



Sulfate reduction results in sulfide formation, which can be toxic to MPB and SRB as well (Karhadkar et al., 1987; Reis et al., 1992).

2.3 Competition between SRB and MPB

SRB and MPB exist in the similar environment and they compete with each other for the organic carbon source. This competition increases with a decrease in the availability of the substrate. The outcome of competition depends on several factors such as:

- COD/SO₄²⁻ ratio
- pH
- Sulfide toxicity
- Hydraulic retention time (HRT)
- Temperature

2.3.1 Effect of COD/SO₄²⁻ Ratio

Methanogenesis and sulfidogenesis can occur simultaneously in an anaerobic digestion process. The intermediate products formed in the process, acetate and hydrogen,

can be used by MPB and SRB, which creates the competition between the two groups. The amount of organic substrate, commonly reported as chemical oxygen demand (COD), provided per unit mass of sulfate (COD/SO₄²⁻ ratio) is an important factor which affects the outcome of competition between SRB and MPB. The organic carbon availability is more commonly measured as COD in domestic and industrial wastewater treatment rather than biochemical oxygen demand (BOD). This could be due to shorter analysis time and better reproducibility of COD analysis than that of BOD analysis. Time required for COD analysis is few hours whereas BOD analysis usually takes 5 or more days.

Sulfate reduction by SRB takes place according to the following equation (Lens et al., 2002).



Each mole of electron is equivalent to 8 g of COD. Theoretically, 64 g of COD is required for 96 g of sulfate reduction. In other words, the ratio of COD/SO₄²⁻ of 0.67 is theoretically sufficient for complete sulfate reduction, however extra amount of organic carbon source needs to be added when MPB competing with SRB for the organic substrate are present. At lower COD/SO₄²⁻ ratios (< 2), its importance increases as less amount of organic carbon is available and hence more is the competition between SRB and MPB for the substrate. At higher COD/SO₄²⁻ ratios, this competition decreases, however addition of more COD increases the overall cost of the process. This is particularly important as acid mine drainage has very low organic content (< 10 mg/L) (Kolmert and Johnson, 2001) and organic substrate needs to be added externally to carry out the biological sulfate reduction process (Gibert et al., 2004). In the study done by Cao et al. (2012) with a decrease in

COD/SO₄²⁻ ratio from 3.03 to 1.3 amount of sulfate reduced increased from 395 mg/L to 700 mg/L, which indicates that SRB activity increases with a decrease in the COD/SO₄²⁻ ratio. Chai et al. (2005) reported sulfate reduction of 90% at COD/SO₄²⁻ ratio 1.45. According to the study done by Choi and Rim (1991), MPB dominated at COD/SO₄²⁻ ratios > 2.7 and SRB dominated at COD/SO₄²⁻ ratios <1.7, whereas there is an active competition between the two groups in the ratio between 1.7 and 2.7. Freese and Stucky (2004) reported dominance of sulfidogenic activity between COD/SO₄²⁻ ratio of 1 to 2. El-Bayoumi et al. (1999) concluded that, COD/SO₄²⁻ ratios less than 1.5 are better for SRB growth than the ratios higher than 2.5. Supporting the studies mentioned above, several other researchers have reported an increase in sulfidogenic activity with a decrease in the COD/SO₄²⁻ ratio less than 2 (Isa et al., 1986b; McCartney & Oleszkiewicz, 1993; Omil et al., 1997a). Even though at lower COD/SO₄²⁻ (< 2) SRB are favored than MPB, presence of MPB makes the biological sulfate reduction less efficient as less amount of organic substrate is available.

2.3.2 Effect of pH

Even though SRB and MPB exist in similar environmental conditions, they have different pH optima. Reis et al. (1992) reported a pH of 6.7 for highest SRB growth in a batch study at 37 °C. In contrast to this observation, O'Flaherty et al. (1998) reported that SRB and MPB have comparable growth rates in the pH range of 7–7.5; above and below this pH range, SRB and MPB are favored, respectively. This study was supported by Visser et al. (1996) who observed that acetoclastic SRB are favored at pH above 7.7 and

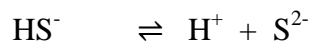
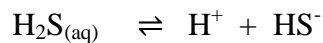
acetoclastic MPB are favored at pH below 6.9. Omil et al. (1998) reported that SRB outcompeted MPB in the pH range 6.85 to 7.75. The maximum pH values SRB and acetoclastic MPB survived in anaerobic batch reactors are 10 and 8.5, respectively (Visser et al., 1996). The pH range of 5 to 8 was suggested by Willow and Cohen (2003) for SRB to survive and grow. Optimal pH range reported by Lopes et al. (2007) for SRB and MPB growth are 6.8-7.2 and 6.4 to 6.8. In general these observations indicate that in higher pH range (7-8) SRB are favored and in lower pH range (6.4-6.9) MPB are favored.

The pH value also has an indirect effect on the competition between SRB and MPB through sulfide toxicity which will be discussed in Section 2.3.3.

2.3.3 Sulfide Toxicity

Many studies have reported the toxic effects of sulfide species (HS^- , $\text{H}_2\text{S}_{(\text{aq})}$ and S^{2-}) on anaerobic bacteria consortia and failure of the anaerobic process eventually. This toxicity is assumed to be mainly caused by the undissociated $\text{H}_2\text{S}_{(\text{aq})}$ molecules (Omil et al., 1997a; Rinzema, 1988). Even though the mechanism of inhibition is not clear, it is proposed that the neutral $\text{H}_2\text{S}_{(\text{aq})}$ molecule can permeate through the cell wall and can denature the protein inside the cell by forming sulfide and disulfide cross-linkage between the polypeptide chain (Lens et al., 1998).

In solution, sulfide exists in three different forms (HS^- , $\text{H}_2\text{S}_{(\text{aq})}$, S^{2-}) because of $\text{H}_2\text{S}_{(\text{aq})}$ dissociation, and the speciation depends on the pH of the solution (Figure 2.3).



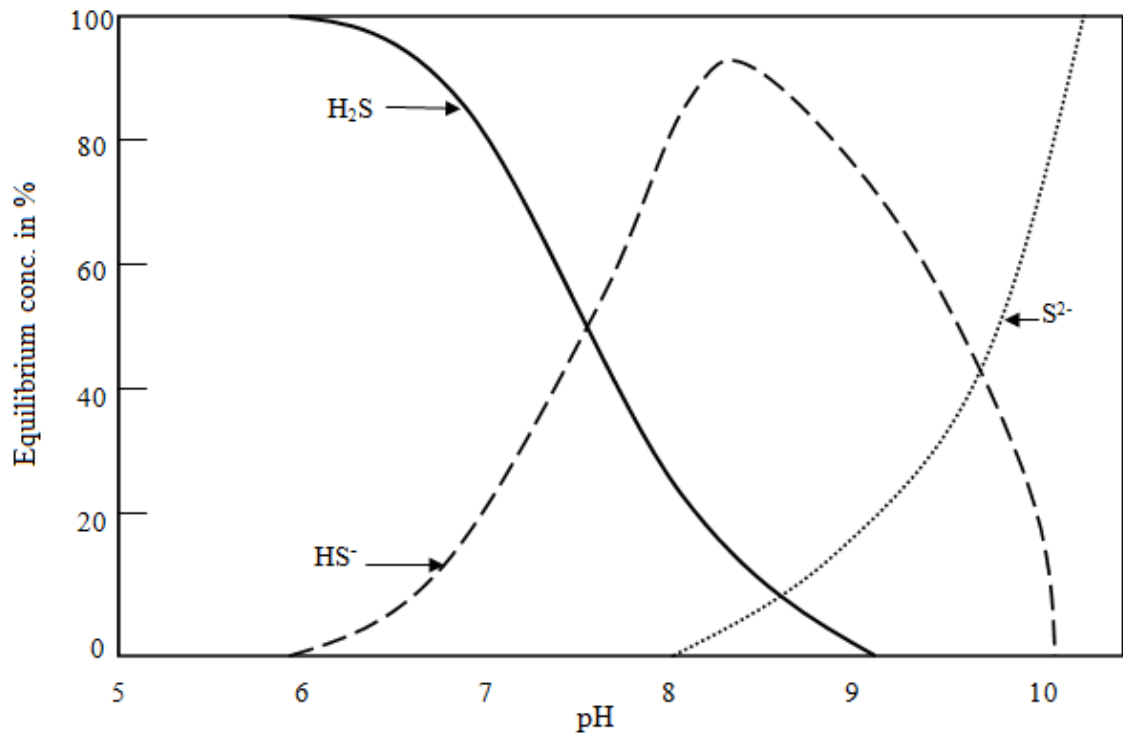


Figure 2.3 Prevalence of sulfide forms at different pH values (Adapted from Rintala and Puhakka, 1994)

The data reported in the literature suggests that MPB are more sensitive to sulfide toxicity than SRB (Omil et al., 1996; Reis et al., 1992; Visser, 1995), however in the study done by McCartney and Oleszkiewicz (1991) SRB were found more sensitive than MPB to an increase in total sulfide concentration. In addition to the undissociated sulfide and pH, sludge characteristics and dissolved sulfide concentrations are also important factors, which affect sulfide toxicity (Maillacheruvu et al., 1993; Parkin et al., 1991; Parkin & Speece, 1983). Granular sludge is found to be more resistant than suspended sludge and the reasons could be i) local pH gradients (Koster et al., 1986) and ii) mass transfer

limitation of sulfide in granular sludge (Overmeire et al., 1994). Sulfide toxicity to MPB in granular sludge is dictated by free sulfide concentration ($\text{H}_2\text{S}_{(\text{aq})}$) in the pH range 6.2-7.2 and by total sulfide concentration when pH is more than 7.2 (Koster et al., 1986). In suspended sludge, however, MPB are affected by free sulfide at low as well as high pH. SRB on the other hand are inhibited by total sulfide concentration in the pH range 7.5 to 9 in both suspended (Oleszkiewicz et al., 1989) and granular sludge (Visser et al., 1996). The data reported in the literature on the concentration of total sulfide and free sulfide causing 50% inhibition of sulfidogenesis and methanogenesis in suspended and granular sludge at different pH is given in Table 2.1 (Lens et al., 1998). The inhibition by sulfide toxicity is found to be reversible by many researchers. Vavilin et al. (1994) reported self-oscillating pattern of growth under hydrogen sulfide inhibition where growth of MPB and SRB decreased with increase in sulfide concentration but both the bacterial groups regained the growth when sulfide concentration was decreased. Similar findings are reported where SRB and MPB activity was restored after H_2S stripping (Oleszkiewicz & Hilton, 1986; Parkin & Speece, 1983; Reis et al., 1992)

2.3.4 Effect of Hydraulic Retention Time (HRT)

HRT in biological wastewater treatment process is a measure of contact time between wastewater and the biomass inside the reactor. It is defined as the average length of time required by any particle to pass through the reactor. In the same amount of time, larger flows can be treated at shorter HRT than at the longer HRT. However, it should be noted

Table 2.1 Unionized sulfide (H₂S) and total sulfide (TS) concentrations causing a 50% inhibition of sulfate reduction methanogenesis (adapted from Lens et al., 1998)

Sludge Type	Substrate	T (°C)	pH	H ₂ S (mg/L)	TS (mg/L)	Reference
Sulphate reduction						
Sludge suspension	Lactate/acetate	35	7.2-7.6	NR	83	McCarteney and Oleszkiewicz (1991)
Sludge suspension	Lactate	35	7.0 8.0	>300 185	NR 2244	McCarteney and Oleszkiewicz (1993)
Sludge suspension	Propionate	35	6.5-7.4 7.7-7.9	100 60	NR NR	Oleszkiewicz et al. (1989)
Sludge suspension	Butyrate	35	6.5-7.4 7.7-7.9	235 >200	NR NR	Oleszkiewicz et al. (1989)
Sludge suspension	Lactate	35	6.5-7.4 7.7-7.9	320 390	NR NR	Oleszkiewicz et al. (1989)
Sludge granule	Acetate	30	7.2-7.4 8.1-8.3	171 57	615 1125	Visser et al. (1996)
Sludge granule	Propionate	30	7-7.5	140	NR	Rinzema and Lettinga (1988)
Methane formation						
Sludge suspension	Acetate	35	6.5-7.4 7.7-7.9	125 100	NR NR	Oleszkiewicz et al. (1989)
Sludge suspension	Lactate/acetate	35	7.2-7.6	NR	240	McCarteney and Oleszkiewicz (1991)
Sludge suspension	Lactate	35	7.0 8.0	100 100	270 1258	McCarteney and Oleszkiewicz (1993)
Sludge granule	Acetate	30	6.4-6.6	246	357	Koster et al. (1986)
Sludge granule	Acetate	30	7.0-7.2 7.2-7.4 8.1-8.3	252 184 38	810 564 590	Visser et al. (1996)

NR – Not Reported

that, very long HRTs may be unfavourable for sludge granulation (Alphenaar et al., 1993) whereas short HRTs may result in washout of biomass (Polo et al., 2006). Literature shows that longer HRT favours SRB more than MPB. Isa et al. (1986a) reported increase in the sulfate reduction from 65% to 98% when HRT was increased from 0.5 to 10 days. Ethanol and acetate was used as a substrate in a high rate reactor in their study. Choi and Rim (1991) obtained the similar results, where they observed an increase in COD and sulfate removal percentage when HRT was increased from 0.5 to 6 days. Polo et al. (2006) reported that a decrease in HRT below 20 h resulted in a washout of biomass and decrease in sulfide production. Selective washout of SRB biomass in high rate reactors at low HRT is attributed to the poor attachment property of SRB (Isa et al., 1986b; Omil et al., 1997c), however Yoda et al (1987) reported comparable attachment properties of SRB and MPB in an anaerobic fluidized bed reactor. This was supported by Alphenaar et al. (1992) and Visser et al. (1993) who reported no difference in attachment property of SRB and MPB in the upflow anaerobic sludge blanket reactor (UASB) reactor.

2.3.5 Effect of Temperature

Reliance of anaerobic process performance on temperature is evident because of its biological nature. An increase in temperature increases microbial growth but after a certain limit the decay rate becomes more than the growth rate, which may severely affect the process. Mesophilic SRB and MPB have similar temperature range and optima (Lens et al. 1998). The study on effect of short term temperature shock in a UASB reactor was done by Visser et al. (1993). They reported that the temperature shock of 45°C did not result in any

effect on SRB or MPB, but temperature shock in the range of 55 to 65°C was detrimental for both the microbial population. After the shock, recovery of SRB was faster than MPB. All the acetate was consumed by MPB prior to the shock, which decreased to 60% and acetate consumption by SRB increased to 40%. Prolonged operation (30 days) at 25°C instead of 35 °C resulted in an increase in COD consumption by SRB from 43% to 80% (Shin et al. 1996). In general, SRB are less sensitive to low and high temperature shocks as compared to MPB, which can be due to the spore forming ability of some species of SRB in a less favourable atmosphere (Widdel 1988)

2.4 Inhibition of Methanogenesis

As stated earlier in Section 2.2.1, a COD/SO₄²⁻ ratio of 0.67 is theoretically enough for complete sulfate reduction. However if MPB are present, part of the COD is consumed by MPB which results in less sulfate reduction. In other words, there is a need to inhibit the methanogenic activity at low COD/SO₄²⁻ ratios for optimum utilization of organic carbon source by SRB. Acid mine drainage has low organic content and therefore it is important to reduce the methanogenic activity to improve the sulfide production for efficient removal of metal content. Researchers have used various techniques to inhibit MPB to develop either an efficient sulfidogenic system or when hydrogen as a product is desired. Physical methods of inhibition include pH treatment and heat treatment. Chemical methods include use of chemicals such as 2-bromo ethane sulphonate (BES), chlorinated methane analogues, long chain fatty acids (LCFA) etc. These inhibition methods and challenges

associated with them are tabulated in Table 2.2. Use of LCFA to inhibit MPB is discussed in detail in Section 2.4.1.

2.4.1 Use of LCFA

The process disturbance in anaerobic digesters because of LCFA presence is reported in several studies (Hanaki et al., 1981; Lalman & Bagley, 2002; Rinzema et al., 1994). The inhibitory effect of LCFA is used for food preservation and for suppressing methane production by supplying it as a dietary supplement to ruminants (Kabara, 1983). LCFA mainly inhibits gram positive bacteria (Nieman, 1954). Most of the strains of MPB are gram positive, whereas most SRB strains are gram negative (Madigan T., 2000; Widdel, 1988). The proposed mechanisms of LCFA inhibition include i) disruption of cell membrane causing leakage of protein and ions (Galbraith.H & Miller, 1973; Greenway & Dyke, 1979) ii) adsorption of LCFA on cell wall affecting permeability and causing problems in transport of the nutrients (Hwu et al., 1998; Rinzema et al., 1994). LCFA are Produced from degradation of lipids and fats, which are abundant in wastewater generated in edible oil refineries, slaughter house and dairy product industries (Kramer, 1971) and are degraded by hydrogen producing acetogens to acetate via a β -oxidation mechanism (Weng & Jeris, 1976).

Various researchers studied the bactericidal effect on MPB of a variety of LCFAs including Oleic acid (OA) (Angelidaki & Ahring, 1992; Galbraith.H & Miller, 1973; Pereira et al., 2004),

Table 2.2 Methods to inhibit MPB

Method	Challenges Associated	Reference
pH treatment	Not effective for long term use as microbial growth is regained when pH stress is relieved	(Duangmanee et al., 2007; Fang & Liu, 2002)
Heat Treatment	Inhibition demonstrated in lab scale experiments but the full scale application is not considered because of the high cost involved	(Oh et al., 2003)
	Highly effective in blocking MPB but because of the high cost and problem of discharge to the environment, full scale application is not feasible	(Oremland & Culbertson, 1992; Scholten et al., 2000)
Acetylene	It also affects several other micro-organisms. In other words, it is a not specific inhibitor. Inhibition is reversible after gassing with nitrogen	(Ahring & Westermann, 1987; Sparling et al., 1997)
Ethylene	Inhibition reported in marine sediments and pure culture but it is found to be reversible as growth is regained when application of the gas is stopped	(Oremland & Taylor, 1975; Schink, 1985)

capric acid (CA) (Rinzema et al., 1994), stearic acid (SA) (Angelidaki & Ahring, 1992) and linoleic acid (LA) (Lalman & Bagley, 2002; Sharma & Biswas, 2010). The inhibitory effect has been shown to increase with increase in number of carbon atoms and carbon double bonds in the LCFA molecule (Lalman & Bagley, 2000). The order of toxicity to MPB found was: LA (18 carbon atoms, 2 carbon double bonds) > OA (18 carbon atoms, 1 carbon double bond) > SA (18 carbon atoms, no carbon double bond). The advantages of using LCFA as a MPB inhibitor are i) they are cost effective and easily available ii) they are degradable and the product of their degradation can be used by other terminal electron acceptors such as SRB (Sharma & Biswas, 2010). Although the use of LCFA for inhibition of MPB has been long recognized and studied, its use to selectively inhibit MPB while maintaining the activity of SRB has only been recently attempted. Sharma and Biswas (2010) studied the effect of LA in batch reactors and reported that, substrate utilization by SRB increased and that by MPB decreased with increase in the LA concentration from 100 to 1000 mg/L. In LA (1000 mg/L) fed cultures 68% more sulfate was reduced than non LA fed cultures. Extending the work of Sharma and Biswas (2010), Biswas (2012) has used LA to successfully divert more organic carbon towards sulfate reduction in a semi-continuous application. In suspended growth semi-continuous stirred tank reactors at LA concentration of 1000 mg/L, COD/SO₄²⁻ ratio of 0.75, and HRT of 40 days, sulfate reduction was shown to increase to almost 99% from about 50% in the control reactor where no LA was added.

2.5 Metal Precipitation

Common chemical methods for removing metals in solution is their precipitation as metal sulfide or hydroxide. Calcium hydroxide (Ca(OH)_2), calcium oxide (CaO) and sodium hydroxide (NaOH) are most commonly used reagents for metal hydroxide precipitation. Even though hydroxide precipitation method has advantages such as low reagent cost and ease of operation, disadvantages such as bulky sludge formation, possibility of resolubilization at higher pH, failure in presence of chelating agents makes this process less attractive (Prasad and Henry, 2003).

Sodium sulfide (NaS), calcium sulfide (CaS) and ferrous sulfide (FeS) are some chemicals used for sulfide precipitation. However chemical sulfide precipitation is not widely used for AMD treatment because of high cost of chemicals (Kaksonen & Puhakka, 2007). The potential of cost effective precipitation of metals by biologically produced sulfide has also been shown by various researchers in a single stage or two stage process described in Section 2.1 (El Bayoumy et al., 1997; Gallegos-Garcia et al., 2009; Kieu et al., 2011; Prasad & Henry, 2009; Velasco et al., 2008). The advantages of sulfide precipitation over hydroxide precipitation are i) metal sulfides have lower solubility than metal hydroxides; ii) better settling properties of metal precipitate formed iii) faster reaction rates iv) selectivity of metal removal (Lewis, 2010). The value of pH plays an important role in the formation of different metal complexes by sulfide precipitation and hydroxide precipitation as well (Sheoran et al., 2010).

CHAPTER III

DESIGN AND METHODOLOGY

The focus of this chapter is to provide details on experimental design and methodology used in this thesis to achieve the stated objectives. Collection of inoculum, substrate composition, operational conditions, start-up of two upflow anaerobic hybrid reactors (UAHR), analytical parameters and methods to quantify these parameters are discussed in this chapter.

3.1 Inoculum Source

The anaerobic mixed culture used in the experiments was obtained from semi-continuous suspended growth reactors. These reactors are referred to as master reactors since, the culture from these reactors was used for the start-up of UAHRs. The master reactors were originally started by a colleague using anaerobically digested sludge from the municipal wastewater treatment plant, Chatham, Ontario (Biswas, 2012). The sludge was grayish black in colour and had a distinctive odour of H_2S .

3.2 Substrate Composition

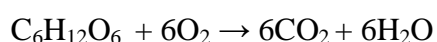
Substrate composition adapted from El-Bayoumy et al. (1997) was used. The substrate composition is given in Table 3.1. This composition was used for maintaining SRB culture in master reactors as well as for the operation of the UAHR reactors for the duration of this study. As glucose is a cheaper source of organic carbon than lactate,

lactate was replaced by glucose. Sodium sulfate was used as a sulfate source. Tap water was used as a source of micronutrients.

Table 3.1 Substrate composition (Adapted from El-Bayoumy et al. 1997)

Name	Chemical formula	Concentration (mg/L)
Sodium sulfate	Na ₂ SO ₄	4500
Glucose	C ₆ H ₁₂ O ₆	2850
Ammonium chloride	NH ₄ Cl	1000
Potassium phosphate monobasic	KH ₂ PO ₄	400
Potassium phosphate dibasic	KH ₂ PO ₄	100

Theoretical oxygen demand (ThOD) of the substrate was calculated according to the following reaction.



Glucose concentration of 180 mg/L is equivalent to 192 mg/L of ThOD which corresponds to 3040 mg/L of substrate ThOD for 2850 mg/L of influent glucose. Glucose is a simple sugar and a very easily oxidizable substrate because of which ThOD and COD of the substrate solution is expected to be the same. Sodium sulfate concentration of 4500 mg/L contributes 3040 mg/L of sulfate (SO₄²⁻), resulting in COD/SO₄²⁻ ratio of 1. As only glucose contributed to the organic carbon in the substrate, total organic carbon (TOC) was measured to calculate the organic carbon removal in the anaerobic digestion process. The substrate TOC and sulfate concentrations were measured using the methods described in

Section 3.7. TOC concentration of 1240 ± 18 mg/L and sulfate concentration of 3040 ± 60 mg/L was the range of substrate composition over the duration of the study.

3.3 Master Reactor Operation

Two 4 L stirred tank reactors (referred as MR1 and MR2), were operated in a semi-continuous mode. In a semi-continuous mode, calculated amount of effluent from the reactor was replaced by the substrate solution every few days. The calculations for amount of substrate fed to the reactor to maintain a certain HRT (e.g. 50 days) is given in the Appendix A. The reactors were operated at room temperature (22 ± 2 °C) and at an HRT of 50 d. The performance of the reactor and SRB growth was monitored by analysing the samples at an interval of 5 days. Data obtained from MR1 and MR2 in the current study is presented in the Appendix B.

3.4 Experimental Setup of UAHR

Figure 3.1 shows the experimental setup of the UAHR, which is a combination of a packed bed reactor and a sludge blanket reactor. The reactor height was 120 cm and internal diameter was 100 mm. The UAHRs had net empty volume of 9.8 L and liquid volume of 8 L. Two identical set ups were made to study the effect of linoleic acid and HRT. The hybrid reactor was developed by introducing the polypropylene pall rings to provide surface area to support the biomass formation. Each setup has the following components:

- Peristaltic pump

- UAHR (Column)
- Connecting tubes
- Gas collection and measurement system

A single pump was used for the recirculation and feeding to the reactor. The recirculation ratio was adjusted to maintain the upflow velocity of 0.35 m/h inside the column. The column was fabricated using PVC and 300 series stainless steel material. The middle 1-foot section was made of stainless steel to use the heating band (tape type by BriskHeat) arrangement to maintain temperature of 35 ± 2 °C inside the reactor. Polypropylene pall

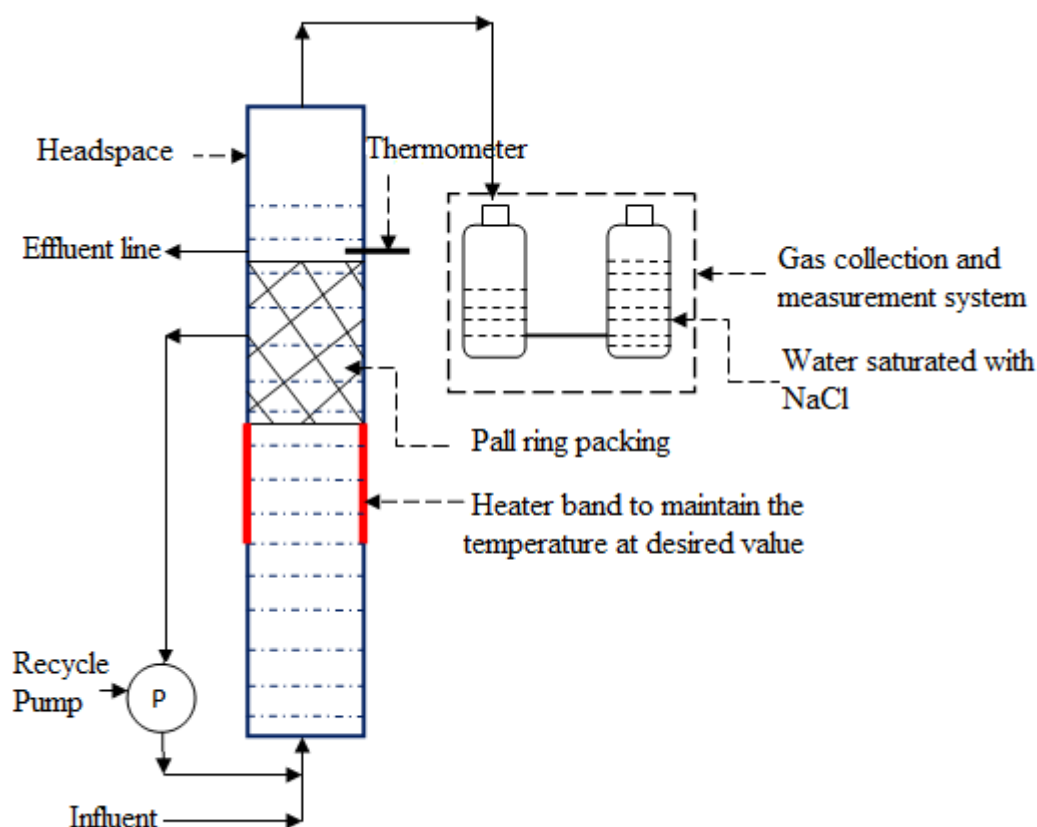


Figure 3.1 Schematic Diagram of Upflow Anaerobic Hybrid Reactor

rings of diameter 25 mm and specific surface area $233 \text{ m}^2/\text{m}^3$ were used as a packing material. Pall rings occupied 20% of the column volume. Digital thermometer was installed for continuous temperature monitoring. Simple liquid displacement method was used for the measurement of gas production during the anaerobic process.

3.5 Operating Procedure of UAHR

The UAHRs were operated in a semi-continuous and an upflow mode. Semi-continuous operation of UAHRs was similar to the operation of the master reactors and it allowed the ease of operation as compared to the continuous mode. The effluent taken out was used for the analyses, which are discussed later in this chapter. The pump tubing was changed periodically to avoid excessive wear and tear. As the HRT was changed during the course of study, amount and frequency of substrate fed varied accordingly (Appendix A).

3.6 Experimental Design

The two UAHRs used in this study were termed as R1 and R2. Both the UAHRs received the same substrate as the master reactors (Table 3.2). The reactor operation was divided into 3 phases.

3.6.1 Phase I- Start-up of UAHRs

Phase I consisted of start-up of the two UAHRs, R1 and R2 at 50 d HRT under identical conditions. The purpose of the start-up phase was to let suspended growth culture

adapt to the new reactor configuration and to check if two UAHRs were showing similar performances in terms of sulfate and TOC reduction. Each reactor was inoculated with 4 L of suspended growth culture from the master reactors. The content of MR1 and MR2 was mixed before the inoculation to UAHR to maintain the similar initial characteristics of culture. The reactors were operated in a semi-continuous mode. Each reactor was then fed 800 mL of substrate (Table 3.1) every fifth day until it achieved liquid volume of 8 L. After this stage 800 mL of the reactor content was replaced every fifth day with the substrate solution to maintain the HRT of 50 days (Appendix A). Both the reactor temperatures were maintained at $35 \pm 2^{\circ}\text{C}$.

3.6.2 Phase II- Effect of LA Treatment and HRT

Phase II involved addition of linoleic acid (LA) dose to R1 and varying of HRT in both the reactors. One time dose of LA was added to the UAHR R1 such that 1000 mg/L concentration was achieved inside the reactor. R2 was used as a control reactor without any LA addition. The hydraulic retention time (HRT) in both the reactor was changed when steady state reactor performance was observed. When properties of the system do not change with time, it is called as a system at steady state. A steady state was assumed to be achieved when variation of all the parameters was within $\pm 5\%$ of the average value for 10 days or more. Phase II corresponds to the first stage of the two stage process mentioned in the Section 2.1 (Figure 2.1). Experimental design of phase II is given in Table 3.2.

Table 3.2 Experimental Design- Phase II

Phase	HRT (day)	Duration (day)	LA dose	
			R1 (LA Treated)	R2 (Control)
II- LA treatment & HRT study	50	50	1000 mg/L	X
	30	50	X	X
	15	22	X	X
	7	22	X	X

X- LA dose not added

3.6.3 Phase III- Metal Precipitation

In phase III potential of metal precipitation, using effluent from the UAHRs called as sulfidogenic liquor (SL) was tested. This phase corresponds to the second stage of the two stage process (Figure 2.1). UAHRs were taken back to HRT of 30 d and 15 d. SL was mixed with the simulated acid mine drainage (SAMD). SAMD as described by Chang et al. (2000) was modified in the current study, as precipitation of only copper was studied. The modified SAMD used in this study contained CaCl_2 , 0.10 g/L; $(\text{NH}_4)_2\text{SO}_4$, 1.00 g/L; KH_2PO_4 , 0.75 g/L; and Na_2SO_4 , 1.48 g/L, $\text{CuSO}_4 \cdot 5\text{H}_2\text{O}$, 8.98 g/L. High concentration of copper (2000 mg/L Cu^{2+}) in the SAMD was used to test the maximum capacity of metal precipitation of biologically produced sulfide. Concentrated sulfuric acid was used to adjust the pH of the SAMD to value 2 before mixing with the SL. AMD and SL were mixed in different ratios. The different AMD: SL volume ratios used were 3:7, 5:5, 7:3 and 9:1. The pH immediately after mixing was measured.

3.7 Analytical Methods

The liquid samples were filtered through glass micro-fiber filter (Whatman 934.AH) and then analyzed for sulfate, total organic carbon (TOC), alkalinity and total volatile fatty acids (VFA).

3.7.1 pH

Immediately after sampling, pH of the effluent was measured. An Oaklon pH meter as per the standard methods (APHA, 1998) used in this method was calibrated for pH buffers 4 and 7 before the analysis. The sample was directly discharged into a beaker containing cleaned pH meter and the reading was noted. The value of pH was used as an indication of the health of the reactor and environmental conditions inside.

3.7.2 Sulfate

Influent and effluent sulfate concentrations were measured using gravimetric method by drying of residue as per Standard Methods: 4500-SO₄²⁻ D (APHA, 1998). This method was selected for its flexibility to analyze the samples with high sulfate concentrations (> 10 mg/L). Barium chloride is used in this method to precipitate the SO₄²⁻ ions and the dried precipitate weight was used to calculate the sulfate ion concentration. Sample volume of 10 mL was used for the analysis. Coefficient of variance less than 2% was accepted.

3.7.3 Alkalinity and Total Volatile Fatty Acids (VFA)

Alkalinity is the capacity of water to neutralise the acid. The Alkalinity and total VFA were determined by direct titration method (DiLallo, 1961). These parameters are indicative of the biochemical environment of the reactors. A sample volume of 10 mL was titrated to pH 4 using 0.01N H₂SO₄ acid to calculate the total alkalinity. The sample pH was adjusted to 3.3-3.5 and it was boiled for 3 minutes. The sample after cooling to room temperature was titrated from pH value 4 to 7 using 0.05 N NaOH for total VFA calculation. VFA concentration was obtained as mg/L of acetic acid. Bicarbonate alkalinity (mg/L as CaCO₃) was calculated by calculating the difference between the total alkalinity and the VFA concentration. As per the procedure, above 180 mg/L acid alkalinity value, correction factor of 1.5 was used to determine the total VFA concentration as acetic acid.

3.7.4 Total Organic Carbon (TOC)

As per Standard Methods: 5310 B (APHA, 1998), Shimadzu TOC-V_{CSH} Total Organic Analyzer was used to measure the total organic carbon in the effluent. The organic carbon was measured by calculating the difference between the total carbon (TC) and inorganic carbon (IC). The samples were acidified below pH 2 before the analysis. Calibration curves for TC and IC were made by injecting standards in triplicates. Coefficient of variance < 2% was accepted. The analysis was performed in duplicates.

3.7.5 Total Dissolved Sulfide (TS)

The total dissolved sulfide was measured by the 4500-S²-C iodometry method as per standard methods (APHA, 1998). The samples were not filtered to avoid the loss of volatile H₂S_(aq) during handling. The samples were pretreated with zinc acetate to remove the interference by sulfite, thiosulfite and other organic compounds that can react with iodine. The analysis was done in triplicates. The coefficient of variation observed was less than 5%.

3.7.6 Gas Production

Gas production was measured using calibrated aspirator bottles by volume displacement arrangement (Biswas 2012). Gas production over a period was measured by calculating the difference between the initial and the final level of the liquid inside the bottles. Bottles were filled with water saturated with NaCl to avoid the dissolution of gases evolved during the anaerobic digestion.

3.7.7 Gas Analysis

The headspace samples were analyzed for hydrogen, methane, carbon di-oxide and hydrogen sulfide gases by Varian 3800 gas chromatograph equipped with thermal conductivity detector. The column used was HP- PLOT/Q (30 m x 0.535 mm x 40 µm film thickness) with carrier gas nitrogen flowing at 1 mL/min. The injector, oven and the detector temperature used were 95 °C, 105 °C, 200 °C, respectively. The calibration curves for all four gases were made with standards in triplicates.

3.7.8 Copper Analysis

After addition of SL to SAMD, the mixture was shook well and the samples were set aside for 3 hours to let the precipitate formed settle down. The supernant from the samples then was filtered through 0.45 μm membrane filter for residual copper analysis in the filtrate. The samples were diluted and the pH was adjusted between 4.6 using either hydrochloric acid or sodium hydroxide depending upon the initial pH of the diluted samples before the analysis. The residual copper was analyzed by USEPA approved Bicinchoninate method (HACH company). Agilent UV spectrophotometer was used for the analysis. Copper peak was detected at 560nm wavelength. This analysis was performed in duplicates. The calibration curve for copper was made with standards in triplicates. Coefficient of variance less than 2% was accepted.

CHAPTER IV

ANALYSIS OF RESULTS

The experiments were divided into 3 phases as stated in Section 3.5. Phase I included start-up of the two upflow anaerobic hybrid reactors (UAHR), R1 and R2. In phase II the effect of the addition of an LA dose to R1 and the effect of varying hydraulic retention time (HRT) in both the reactors was investigated and in phase III metal removal efficiency of biologically produced sulfide was tested. Two master reactors were operated at an HRT of 50 days in a semi-continuous mode. The $\text{COD}/\text{SO}_4^{2-} = 1$ was maintained with influent SO_4^{2-} and COD concentration 3040 mg/L. The data obtained from the master reactors is presented in Appendix B. The suspended growth culture from the master reactors was used for start-up of the two UAHRs. The UAHR setup used in the study is described in the Section 3.6.

4.1 UAHR Start-up- Phase I

As stated earlier in Section 3.5.1, the purpose of the start-up phase was to let suspended growth culture adapt to the new reactor configuration and to check if two UAHRs were showing similar performances in terms of sulfate and TOC removal. Initially R1 and R2 had different sulfate reduction efficiencies as observed in Figure 4.1(a). An average effluent sulfate concentration of 1220 mg/L in R1 and 1030 mg/L in R2 was seen in the initial 25 days of operation. This difference in sulfate removal could be because of the temperature fluctuation in the reactor R1. The temperature in R1 fluctuated between 22 ± 2 °C from day 4 to 8 due to heater band failure. R2 was maintained at 35 ± 2 °C. Even

though R1 had higher effluent sulfate concentration initially, it started decreasing slowly after the reactor temperature was brought back to 35 ± 2 °C. As the biomass started developing an increase in sulfate reduction was observed indicated by the decline in effluent sulfate concentration and an increase in the sulfide levels in both the reactors (Figure 4.1(b)). Total organic carbon (TOC) removal efficiency improved over a period as seen in Figure 4.1(c). VFA levels in both the reactors were similar except between 10 to 35 days (Figure 4.2(a)). Bicarbonate alkalinity increased with an increase in the sulfate reduction (Figure 4.2 (b)). It changed from 1200 mg/L to 2000 mg/L in a period of 50 days in both the reactors. The pH value in the reactors remained in the range 7-7.1 (Figure 4.2(c)).

From day 40 to 50, similar performance was achieved in both the reactors. The effluent TOC levels of 45 ± 8 mg/L, the sulfate levels of 908 ± 45 mg/L, the VFA levels of 171 ± 10 mg/L and sulfide levels of $270 \text{ mg/L} \pm 15 \text{ mg/L}$ were observed in both the reactors in last 10 days.

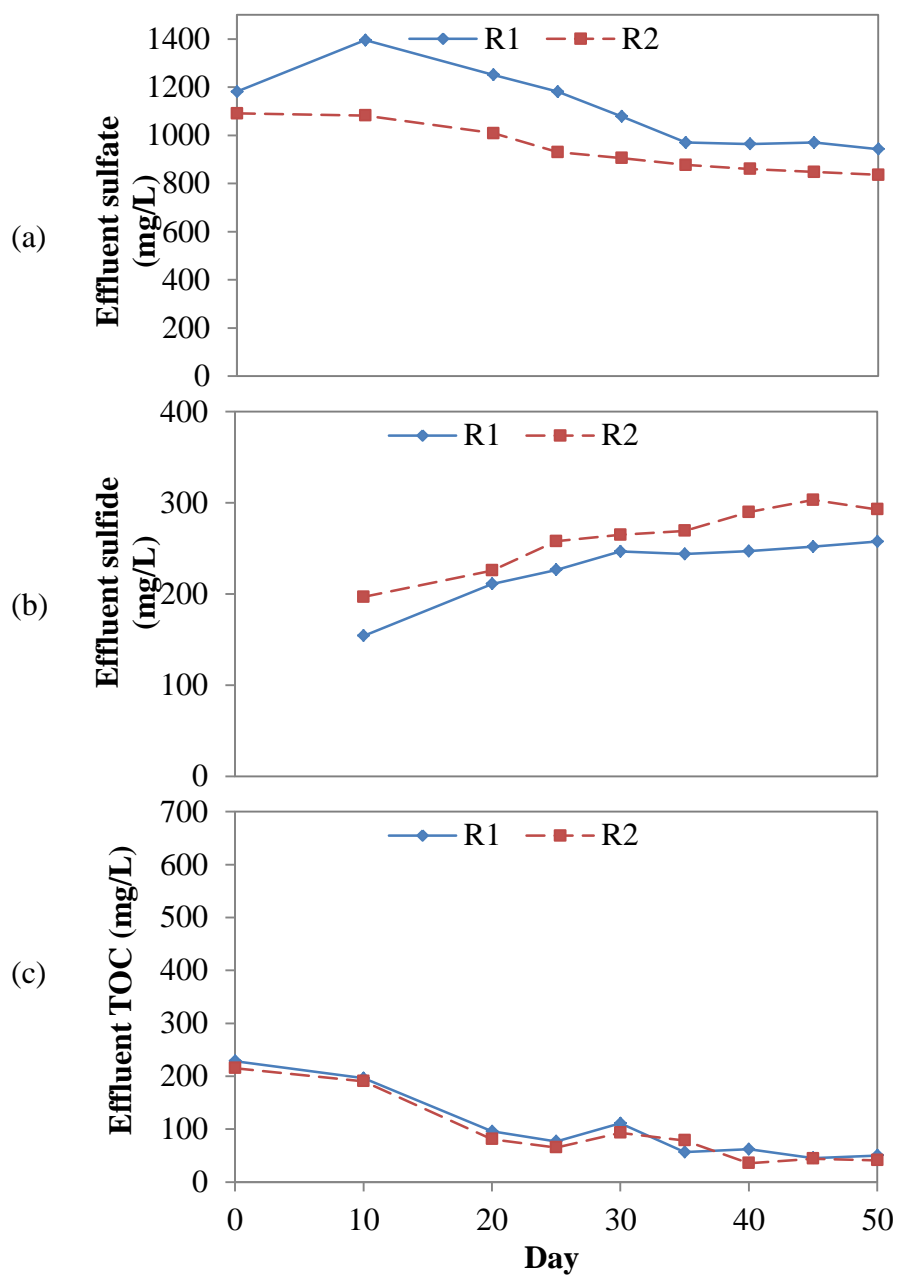


Figure 4.1 Variation of (a) effluent sulfate, (b) effluent sulfide and (c) effluent TOC with time at 50 d HRT

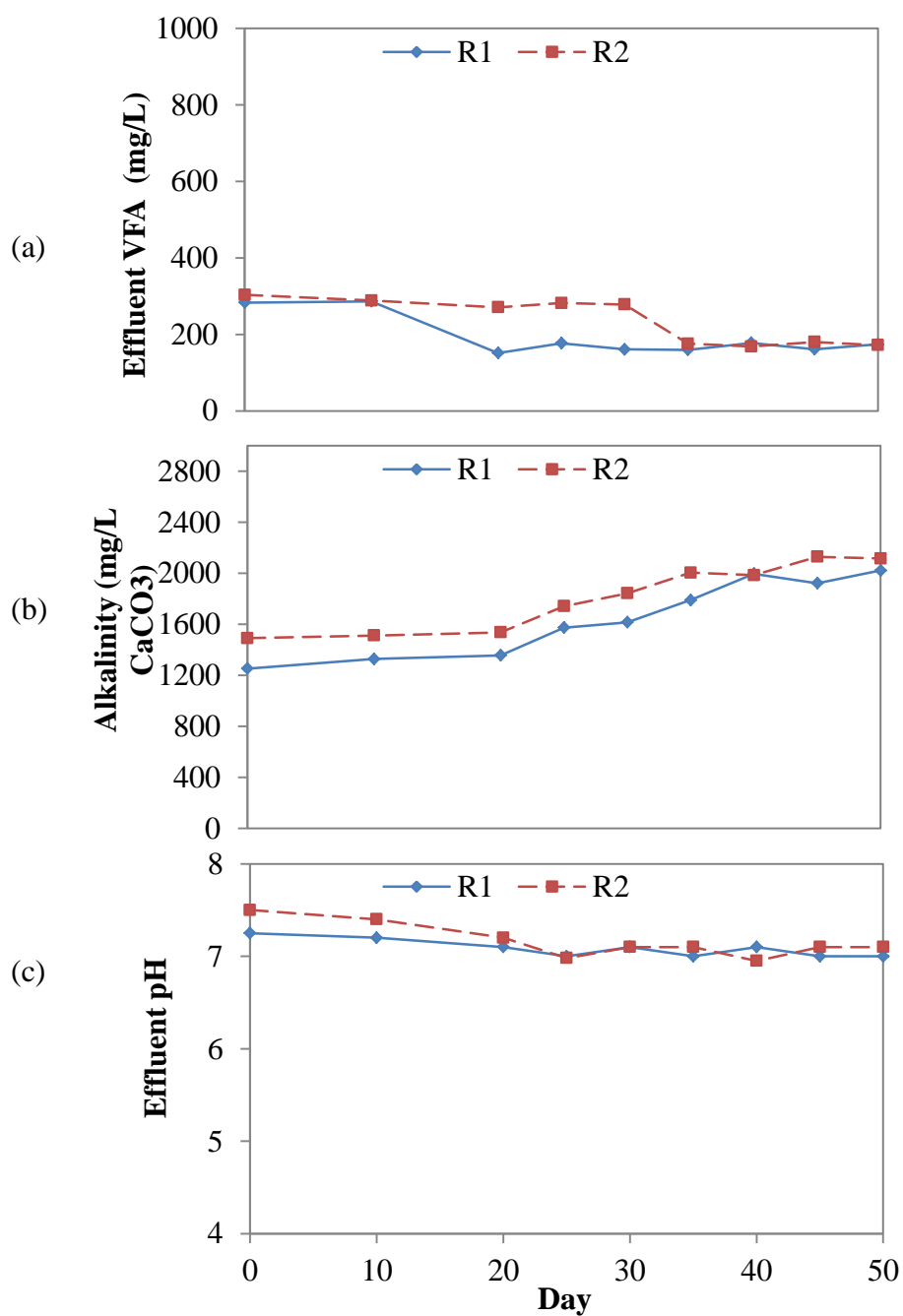


Figure 4.2 Variation of (a) effluent VFA, (b) effluent alkalinity and (c) effluent pH with time at 50 d HRT

4.2 LA Treatment and HRT Study- Phase II

This phase consisted of a series of experiments to observe the effect of linoleic acid (LA) on the process performance of reactor R1 at 50 d HRT and then effect of varying HRTs on the process performance of the LA treated reactor (R1) and the control reactor (R2).

4.2.1 Process Performance at 50 d HRT

After 50 days of operation in start-up phase, both the reactors showed similar performance in terms of sulfate reduction, sulfide production and TOC reduction. At this stage, one time dose of LA was added to the reactor R1 whereas R2 was selected as a control reactor without any LA addition. LA was added such that 1000 mg/L concentration was obtained inside the reactor. The results of this stage are shown in Figures 4.3 and Figure 4.4

4.2.1.1 Reactor R1 (LA Treated)

After addition of LA to the reactor, no significant change in the sulfate reduction was observed in initial 5 days. This lag of 5 days can be attributed to the time required by SRB to get acclimated to the LA dose in the reactor. Similar lag of 10 days in sulfate reduction was observed by Biswas (2012) when 500 mg/L of LA dose was added in a suspended growth reactor. After the lag of 5 days, sharp decrease in the effluent sulfate concentration was observed (Figure 4.3(a)). From day 60 to 80, sulfate level decreased from 900 mg/L to 60 mg/L. Similar results were obtained by Biswas (2012) where the

addition of a 1000 mg/L LA dose resulted in a rapid decrease in effluent sulfate levels from ~ 1000 to 50 mg/L within 20 days in the semi-continuous suspended growth system.

Effluent sulfide concentration increased with a decrease in the effluent sulfate concentration (Figure 4.3(b)). From day 30-90, sulfide level increased from 320 to 430 mg/L. After LA addition, sudden increase in the TOC (Figure 4.3(c)) and VFA (Figure 4.4(a)) levels were observed. About 420 mg/L of TOC increase was observed after addition of 1000 mg/L of LA in 5 days. The elevated TOC concentration noted in R1 could be due to the two reasons i) contribution of LA towards TOC, ii) organic carbon not consumed because of possible inhibition of MPB. Increase in TOC in initial 10 days can be attributed to both the reasons mentioned above. The LA dose was added only one time on day 50. Slow decrease in TOC over the next 30 day could be due to the gradual wash out of LA. Even though the slow decrease was observed till day 95, TOC concentrations were still higher than what it was at the start-up phase (45 ± 8 mg/L) which could be possible due to inhibition of MPB. Inhibition of MPB can also be supported by an increase in VFA level from 170 mg/L to 670 mg/L over a period of 50 days (Figure 4.4(a)). The reactor pH dropped only slightly from 7.0 to 6.9 after LA addition which can be attributed to an increase in the VFA levels (Figure 4.4(c)). Gradual increase in alkalinity from 1900 to 2850 mg/L was observed over a period of 50 days (Figure 4.4 (b)), which can be attributed to an increase alkalinity production due an increase in sulfate reduction.

4.2.1.2 Reactor R2 (Control)

The control reactor R2 did not receive any LA dose throughout the experimental period. The effluent sulfate concentration continued to decrease slowly in the control reactor until day 90 as shown in Figure 4.3(a). This could be attributed to the slow growth of SRB biomass inside the reactor. The sulfide concentration increased with an increase in the sulfate reduction (Figure 4.3(b)). TOC removal was not affected in the control reactor till day 90. From day 75-90, TOC concentration as low as 18 mg/L was observed which then increased to 85 mg/L from day 90-100 (Figure 4.3(c)). This decrease in TOC removal can be attributed to sulfide toxicity to MPB. Severity of the sulfide toxicity was not enough to inhibit MPB to a considerable extent as MPB activity was still evident by low TOC (< 90 mg/L) and VFA (< 170 mg/L) levels. Total sulfide concentration of 630 mg/L is reported by O'Flaherty et al. (1998) for 50% methanogenic inhibition of lab scale sulfate adapted sludge at pH 7.2. The pH level in the control reactor remained in the range 7.0 ± 0.1 (Figure 4.4(c)). The alkalinity increased from 2050 mg/L to 2950 mg/L with the increase in sulfate reduction (Figure 4.4(b)).

4.2.1.3 Effect of LA Treatment – Steady State

On average 98% and 94% sulfate reduction and 480 mg/L and 450 mg/L of average sulfide production was achieved in R1 and R2 reactor respectively under the steady state condition. The effect of LA was clearly seen in R1 by an increase in the VFA level and decrease in the TOC removal efficiency. Significant increase in VFA levels to $669 \text{ mg/L} \pm 5\%$ was an indication of methanogenic inhibition. Increase in VFA levels as

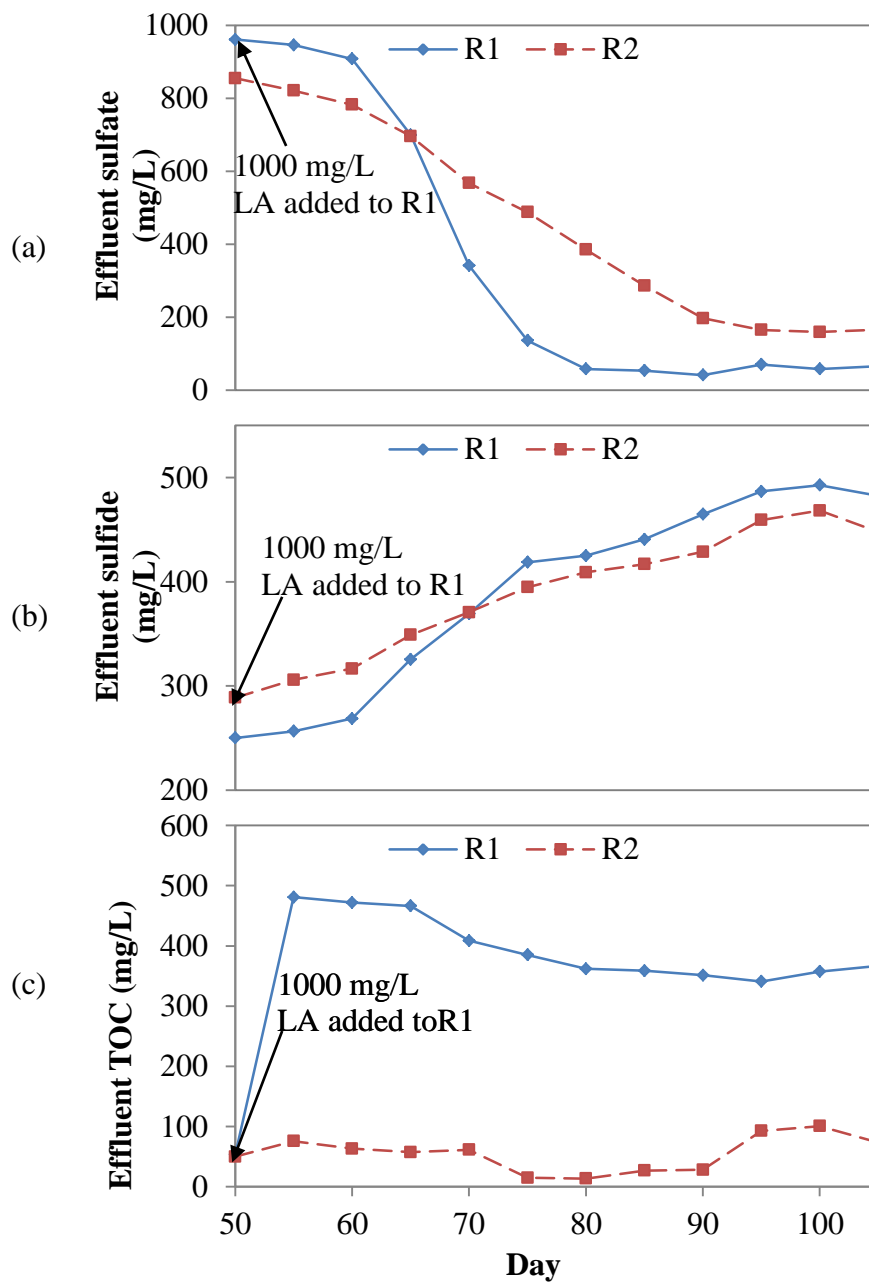


Figure 4.3 Variation of (a) effluent sulfate, (b) effluent sulfide and (c) effluent TOC with time at 50 d HRT after the LA treatment

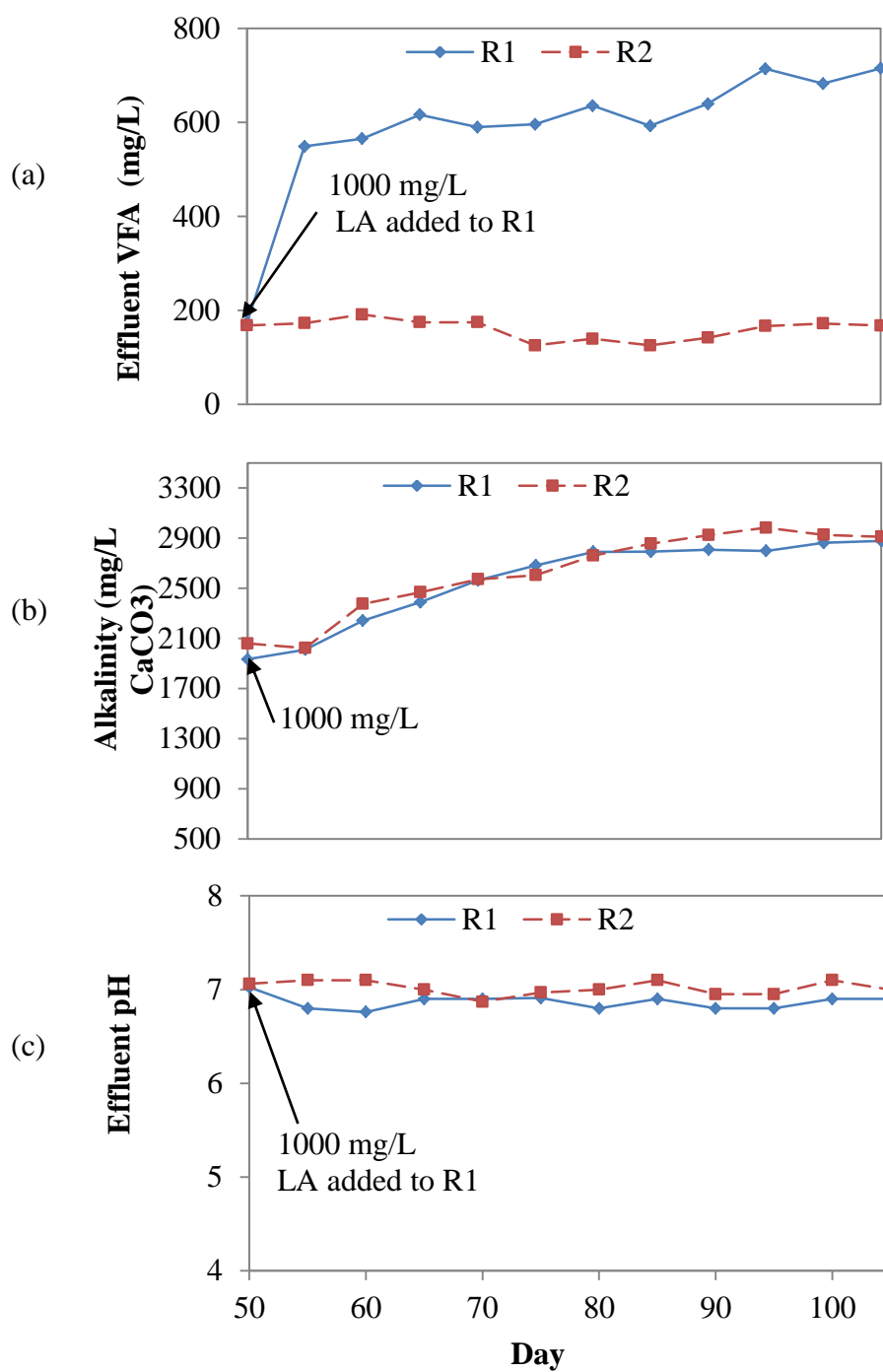


Figure 4.4 Variation of (a) effluent VFA, (b) effluent alkalinity and (c) effluent pH with time at 50 d HRT after the LA treatment

an effect of LA dose was also reported by Biswas (2012). VFA accumulation of about 1450 mg/L over the period of 200 days of operation at $\text{COD}/\text{SO}_4^{2-}=0.75$ is reported. About 500 mg/L increase in VFA was observed compared to start-up phase after addition of LA in R1. This increase corresponds to ~200 mg/L of TOC. In the LA treated reactor R1, 300 mg/L increase in TOC was observed in last 20 days of operation. The difference of ~ 100 mg/L TOC could be through the contribution of other products formed, such as organic acids, alcohols, and any residual LA that remained in the reactor.

In the reactor R2, TOC removal was maintained at 95% but sulfate reduction increased to 94.3%, which indicates that more TOC was consumed by SRB than MPB as compared to the start-up phase. VFA levels in the control reactor after 100 days of operation ($165 \text{ mg/L} \pm 5\%$) remained the same as in the start-up phase ($171 \text{ mg/L} \pm 5\%$). Yoda et al. (1987) reported the similar dominance of SRB in the biofilm in acetate limiting conditions. They attributed this dominance to lower half velocity constant K_s of SRB than MPB ($K_{s(\text{SRB})}=9.5 \text{ mg acetate/L}$, $K_{s(\text{MPB})}= 32.8 \text{ mg acetate/L}$).

4.2.2 Process Performance at 30 d HRT

The results of 30 d HRT stage are shown in Figures 4.5 – 4.6. Gas analysis was started at this stage. Because of the problems in the quantification of gases produced, methane and hydrogen sulfide gases are reported only in terms of presence or absence. The results of gas analysis are presented in Appendix E.

4.2.2.1 Reactor R1 (LA Treated)

The change in the HRT caused an increase in effluent sulfate level in first 10 days followed by a gradual decrease (Figure 4.5 (a)). A decrease in sulfide concentration (480 mg/L to 460 mg/L) with an increase in effluent sulfate level (70 mg/L to 200 mg/L) was observed between day 105 and 115 (Figure 4.5(b)). The decrease in the sulfate reduction and sulfide production in the initial 10 days occurred because of the sudden change in HRT. Such behavior of high rate reactor as a response to a change in HRT has also been reported by Kaksonen et al. (2004).

After one hydraulic turnover at 50 d HRT, it was assumed that LA was washed out from the reactor and it did not contribute to the TOC at 30 d HRT stage. Increase in TOC in first 10 days after the HRT change was due to a decrease in sulfate reduction. VFA level increased to 790 mg/L in the initial 10 days (Figure 4.6(a)), which can be attributed to the same reasons as for TOC increase. After 10 days of operation, VFA levels ranged from 680 mg/L to 735 mg/L in a period of 45 days. Increase in VFA concentration caused a slight decrease in the reactor pH from 6.8 to 6.6 during this period (Figure 4.6(a)). Bicarbonate alkalinity concentration fluctuated between 2550 mg/L and 2300 mg/L (Figure 4.6(b)). Breakdown of VFAs generates alkalinity. The decrease in the alkalinity can be attributed an increase in the VFA levels in the reactor.

4.2.2.2 Reactor R2 (Control)

Three days after the HRT was changed, on day 108, leakage occurred in the pump tubing of the reactor. About 800 mL of the reactor content was lost. This content was replaced by

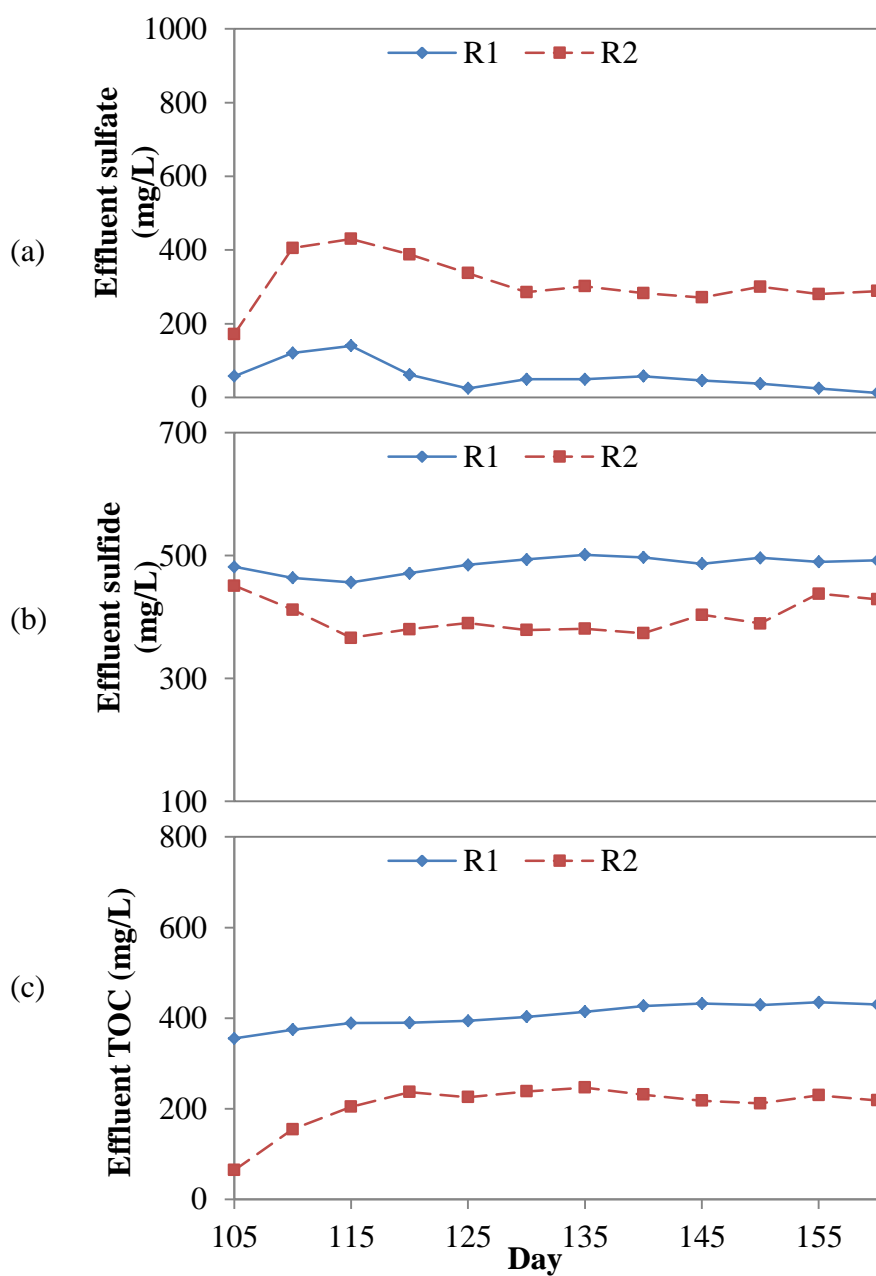


Figure 4.5 Variation of (a) effluent sulfate, (b) effluent sulfide and (c) effluent TOC with time at 30 d HRT

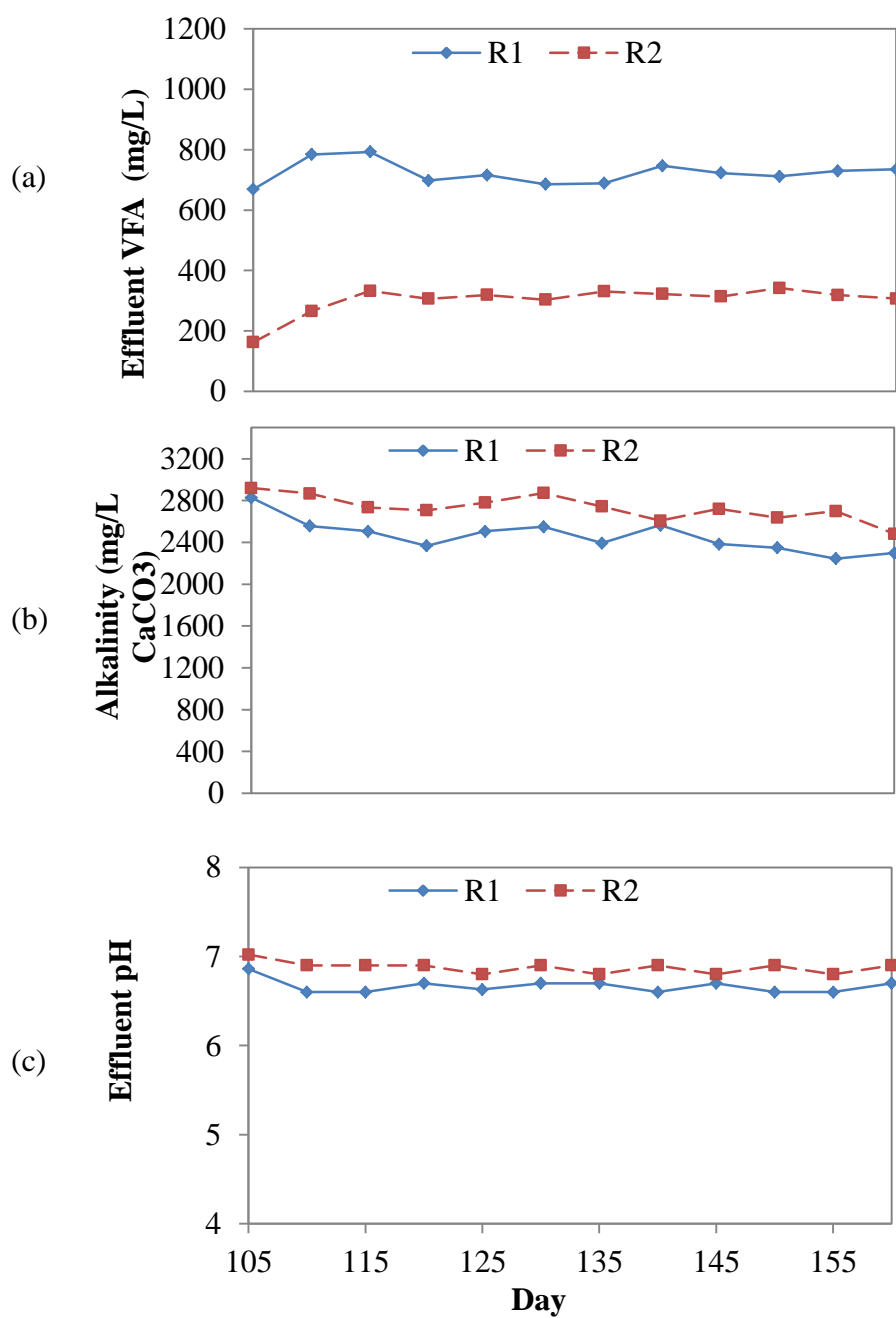


Figure 4.6 Variation of (a) effluent VFA, (b) effluent alkalinity and (c) effluent pH with time at 30 d HRT

the content of the master reactor and the operation was resumed. Sulfate reduction in the reactor decreased during the initial 10 days indicated by an increase in the effluent sulfate concentration (Figure 4.5(a)). This increase could be because of the loss of biomass due to leakage as well as a decrease in HRT. The sulfate level in the effluent increased to 430 mg/L during this period. After 10 days, however the effluent sulfate level gradually decreased till day 145. Sulfide concentration decreased to 366 mg/L in 10 days because of the leakage and the decrease in the sulfate reduction. Improvement in the sulfide production was observed from day 135 to 160. Similar trends as in the effluent sulfate level were observed in TOC (Figure 4.5(c)) and VFA (Figure 4.6(a)) levels. Gradual increase in TOC level was observed until day 120. It ranged between 200 mg/L to 230 mg/L over the next 40 days. The VFA level increased to 330 mg/L after 30 days of operation, which was accompanied by lowering of pH from 7.1 to 6.9 (Figure 4.6(c)). The bicarbonate alkalinity decreased from 2850 mg/L to 2640 mg/L in this stage (Figure 4.6(b)).

4.2.2.3 Effect of LA Treatment and Change in HRT– Steady State

After 25 days of operation, sulfate levels stabilized at an average concentration of 30 ± 10 mg/L in R1. Almost complete sulfate reduction (99%) was obtained in last 15 days of operation. At steady state, effluent sulfide concentration of 492 ± 14 mg/L was obtained. Average TOC removal of 65% was achieved in last 25 days of operation. Theoretically, 66% of TOC is required for 99% sulfate removal, which indicates that TOC removal was accompanied only by SRB and MPB activity was eliminated from the reactor. This can

also be supported by an absence of methane gas in the headspace (Appendix E). Day 135 onwards, methane gas was not detected in R1 headspace. VFA concentration in the reactor increased to average 725 ± 15 mg/L in the last 30 days of operation. A complete sulfidogenic system was developed at this stage.

In the reactor R2, effluent sulfate concentration stabilized at 285 ± 13 mg/L in last 30 days, which is a 91% sulfate reduction. An effluent sulfide concentration of 420 ± 20 mg/L was obtained. The reactor pH fluctuated between 6.9 and 6.8. TOC removal efficiency decreased to 82% as compared to 95 % TOC removal previous 50 d HRT phase in the control reactor R2. Free H_2S concentration of 250-280 mg/L was obtained which may have resulted in inhibition to MPB and hence a decrease in TOC removal. The decrease in TOC removal with an increase in the free dissolved sulfide levels is shown in various studies (O'Flaherty & Colleran, 1999; Omil et al., 1996; Sabumon, 2008). Koster et al (1986) reported 50% inhibition of MPB at 246-252 mg/L free H_2S concentration in the pH range 6.4-7.2 was. In the LA treated reactor, free sulfide concentration was in the range 320-370 mg/L. This sulfide concentration was not found inhibitory to SRB however; it is possible that any MPB remained in the reactor after the LA inhibition, got inhibited by the sulfide toxicity.

4.2.3 Process Performance at 15 d HRT

The HRT was changed from 30 d to 15 d when steady state sulfate reduction and TOC reduction was observed. The results of 15 d HRT are shown in Figure 4.7 and Figure 4.8.

4.2.3.1 Reactor R1 (LA Treated)

The sulfate reduction efficiency decreased with the change in HRT indicated by an increase in the effluent sulfate concentration (Figure 4.7(a)). The sulfate level increased from 35 mg/L to 256 mg/L within 5 days. In next 10 days, gradual improvement in the sulfate reduction was observed as shown by the decrease in effluent sulfate concentration. Sulfide concentration declined to 440 mg/L from 490 mg/L in the initial 5 days followed by the gradual increase as shown in Figure 4.7(b). Effluent TOC concentration also increased with a decrease in the sulfate reduction. These changes in sulfate reduction and TOC removal efficiencies can be explained by an increase in VFA level to 926 mg/L and subsequent drop in the reactor pH to 6.4 in starting 10 days of this phase. A similar phenomenon of decrease in sulfate reduction due to VFA accumulation in an LA treated reactor at COD/SO₄²⁻ of 0.75 is also reported by Biswas (2012). Bicarbonate alkalinity of 1000 mg/L was added in the feed from day 162 to 166 to improve the pH of the reactor. After the addition of alkalinity, the pH inside the reactor changed to 6.6 (Figure 4.8(c)). The effluent sulfate concentration gradually decreased from day 164 to 174 and stabilized thereafter. The bicarbonate alkalinity ranged between 2000 mg/L to 2120 mg/L in this stage (Figure 4.8(b)).

4.2.3.2 Reactor R2 (Control)

The reactor pH decreased to 6.4 immediately after the change in HRT. Bicarbonate alkalinity of 1000 mg/L in the form of NaHCO₃ was added to the feed from day 162 to 166 which changed the reactor pH to 6.6 ± 0.1. The decrease in HRT to 15 d

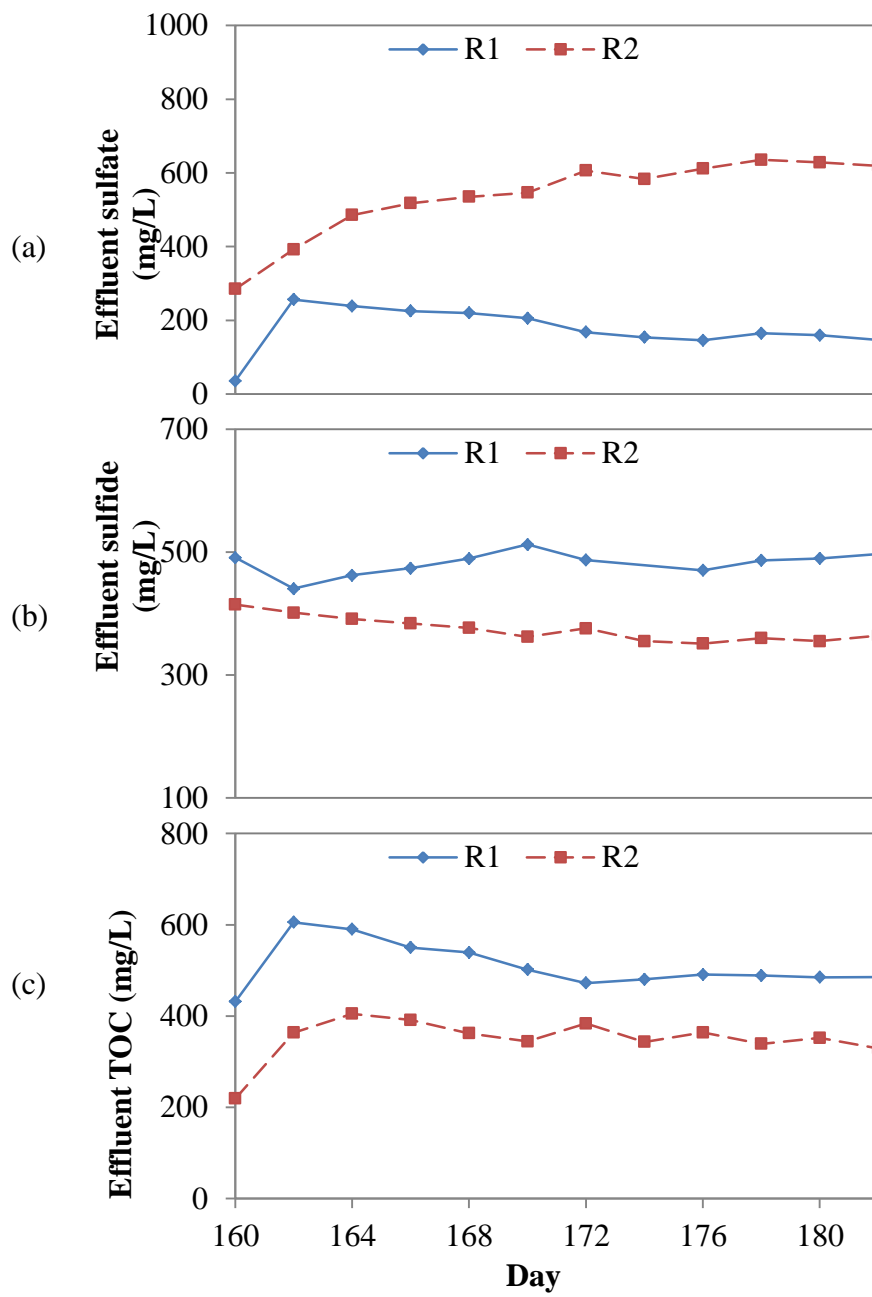


Figure 4.7 Variation of (a) effluent sulfate, (b) effluent sulfide and (c) effluent TOC with time at 15 d HRT

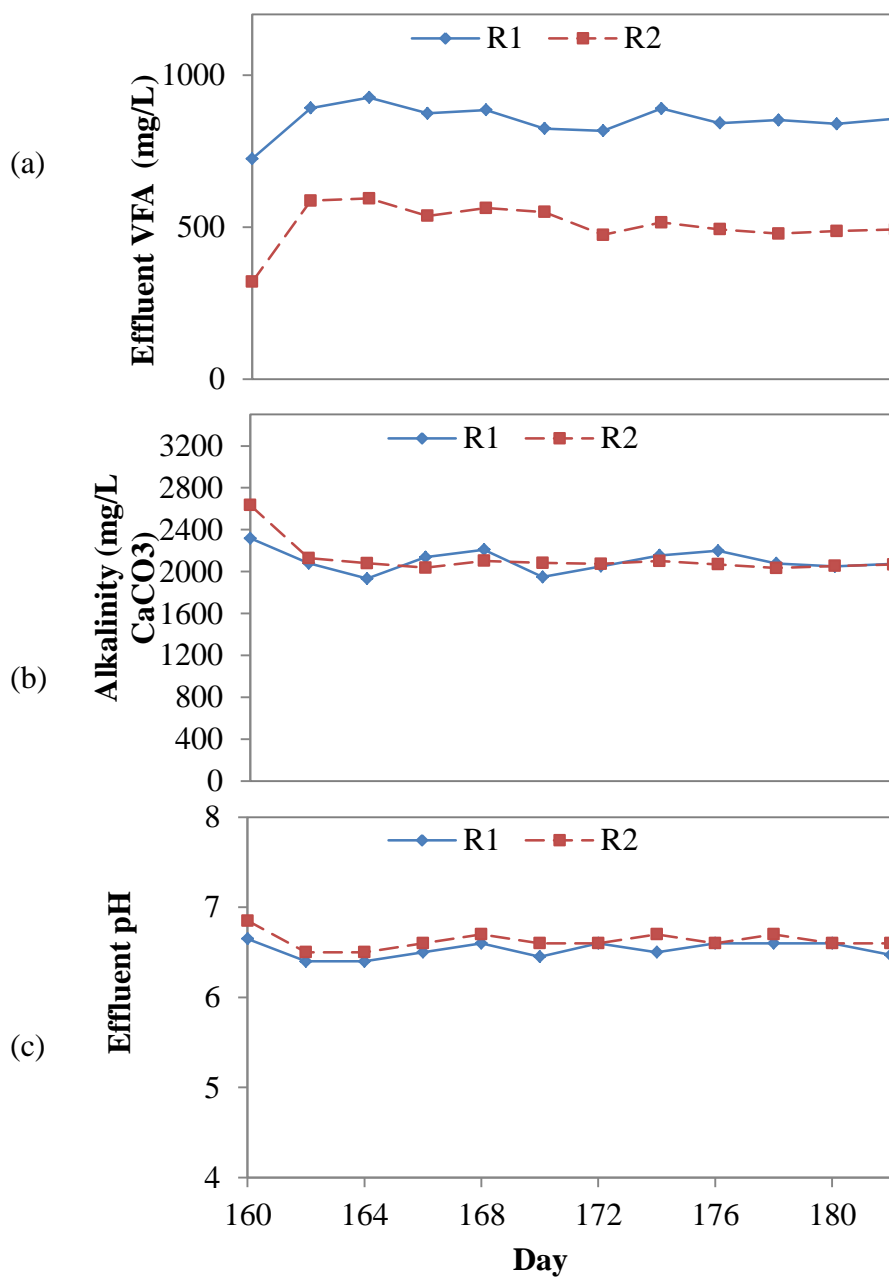


Figure 4.8 Variation of (a) effluent VFA, (b) effluent alkalinity and (c) effluent pH with time at 15 d HRT

adversely affected the sulfate reduction. The effluent sulfate concentration showed increasing trend until day 172. It increased to 606 mg/L and stabilized thereafter as shown in Figure 4.7(a). Sulfide production decreased with a decrease in the sulfate reduction. From day 160 to 170, sulfide concentration decreased from 414 to 362 mg/L (Figure 4.7(a)). The TOC and the VFA levels also increased with the change in HRT as shown in Figure 4.7(c) and 4.8(a). TOC increased from 220 mg/L to 400 mg/L in 10 days followed by a gradual decrease until day 170. From day 172 to 180, TOC ranged between 360 mg/L to 330 mg/L. VFA level also increased in the initial 10 days followed by a gradual decrease and stabilization. The decrease in pH was due to the increase in the VFA level to 595 mg/L immediately after the HRT change. VFA levels ranged between 595 to 475 mg/L in this stage. Bicarbonate alkalinity ranged between 2000-2100 mg/L.

4.2.3.3 Effect of LA Treatment and HRT Change– Steady State

At 15 d HRT, average 95% and 80% sulfate reduction was achieved in R1 and R2 respectively at the steady state. It took almost one hydraulic turnover (12 days) for the reactor performance to stabilize. A decrease in sulfate reduction in R1 (from 99% to 95%) and R2 (from 91% to 80%) was observed as the HRT was decreased from 30 to 15 days. In both the reactors, this decrease could be attributed to biomass washout and an increase in VFA due to the sudden decrease in the HRT. In R1 biomass washout affect was not observed as strongly as in R2 as seen by more decline in sulfate reduction in R2 than R1. This could be possible because of the complete sulfidogenic system developed in R1 and washout of biomass did not decrease the SRB population to the extent it did in R2. Similar

results as in the case of control reactor are reported in the literature where lower sulfate reduction was achieved at shorter HRT (Isa et al., 1986a; Neculita et al., 2008; Omil et al., 1997b; Sunil Kumar et al., 2007). Isa et al. (1986) and Omil et al. (1997) attributed this decrease to wash out of SRB population due to its poor attachment properties. TOC (average 486 mg/L in R1 and 345 mg/L in R2) and VFA (856 mg/L in R1 and 493 mg/L in R2) concentrations in R2 were lower than in R1, which can be attributed to MPB activity in R2. Methane gas was not detected in the headspace of R1 (Appendix E) at this stage also, which suggests MPB activity was not recovered in R1 even after the washout of LA.

4.2.4 Process Performance at 7 d HRT

Results of 7 d HRT are presented in Figure 4.9 and Figure 4.10. This stage continued from 182 d to 204 d.

4.2.4.1 Reactor R1 (LA Treated)

The change in HRT caused the reactor pH to drop to 6.1 from 6.6 (Figure 4.10(c)). From day 184 to 188, 1500 mg/L of bicarbonate alkalinity in the form of NaHCO_3 was added in the substrate solution to increase the pH of the reactor. This dose was increased to 2000 mg/L for all the next feedings as pH increased only to 6.3 in 4 days. The addition of more alkalinity increased the effluent pH to 6.5-6.6. Significant decrease in sulfate reduction occurred, shown by the increase in the effluent sulfate level from 155 mg/L to 600 mg/L in 4 days (Figure 4.9(a)). As observed in previous different HRTs this increase in effluent sulfate concentration was followed by a gradual decrease. Effluent

sulfide concentration decreased to 387 mg/L with the decline in sulfate reduction in the initial 4 days, followed by slow improvement in sulfide production (Figure 4.9(b)).

Decrease in the sulfate reduction was accompanied by an increase in the effluent TOC concentration from day 182 to 186 (Figure 4.9(c)). This can be attributed to the similar reason of VFA built up in the reactor as the previous stage at 15 d HRT. The VFA concentration varied between 1000 mg/L and 1130 mg/L over a period of 22 days (Figure 4.10(a)). With an increase in VFA concentration, bicarbonate alkalinity dropped in the reactor. It ranged between 1400 to 1600 mg/L of CaCO_3 during the stage of 7 d HRT (Figure 4.10(b)).

4.2.4.2 Reactor R2 (Control)

The reactor pH decreased to 6.2 after the HRT change (Figure 4.10(c)). Bicarbonate alkalinity of 1500 mg/L was added to maintain the reactor $\text{pH} \geq 6.6$ throughout the 7 d HRT period. The addition of alkalinity increased the pH to 6.6. The immediate effect of the HRT change to 7 d on the sulfate reduction was also observed in the control reactor. The effluent sulfate concentration showed an increasing trend till day 196. The sulfate concentration of 1295 mg/L was observed on day 196 (Figure 4.9 (a)). The effluent sulfide level started declining with a decrease in the sulfate reduction as shown in Figure 4.9(b). Effluent TOC (Figure 4.9(c)) and VFA (Figure 4.10(a)) levels continued to increase till day 194. TOC values ranged between 340 mg/L – 460 mg/L and VFA values ranged between 500 mg/L – 600 mg/L from day 182 to 194. The bicarbonate alkalinity ranged between 1500 to 1620 mg/L during this stage (Figure 4.10(b)).

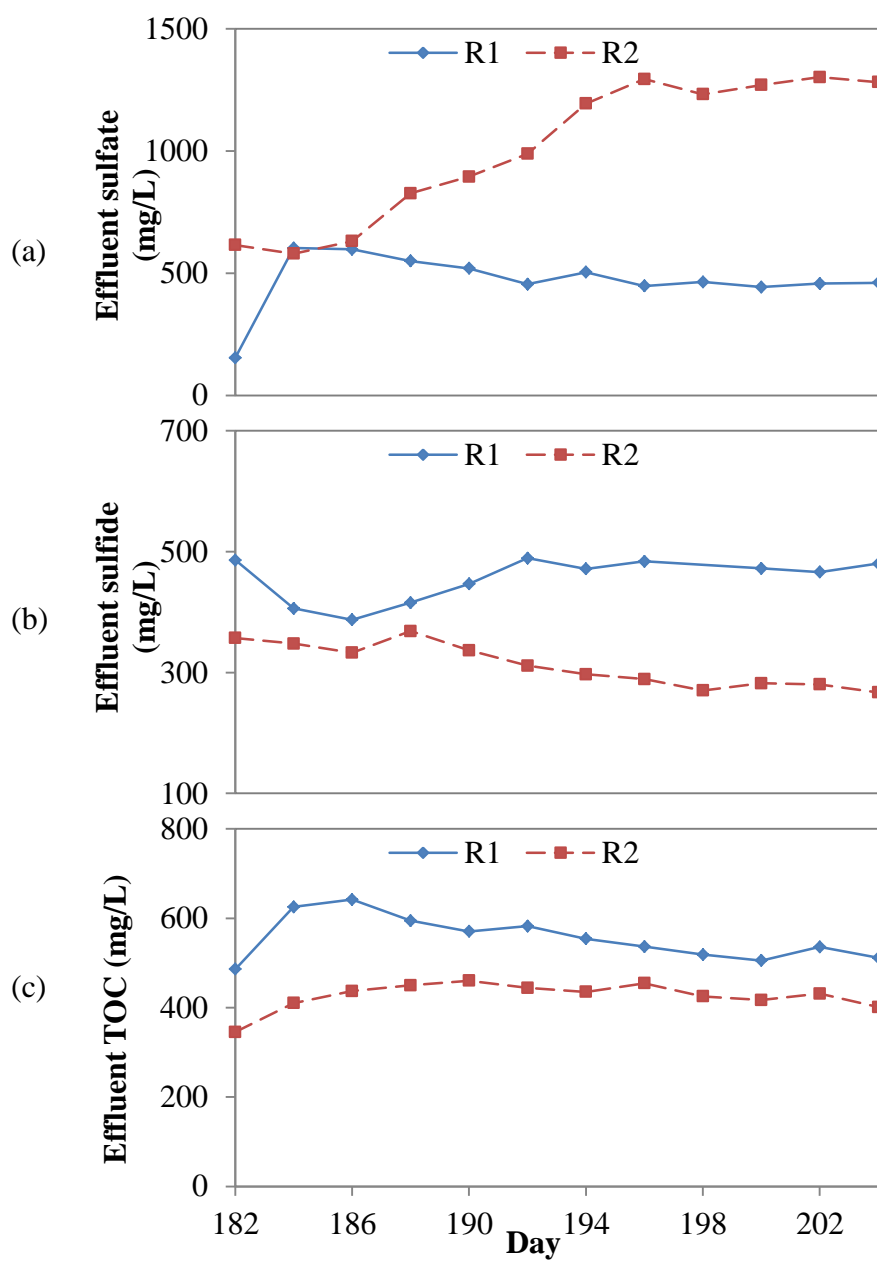


Figure 4.9 Variation of (a) effluent sulfate, (b) effluent sulfide and (c) effluent TOC with time at 7 d HRT

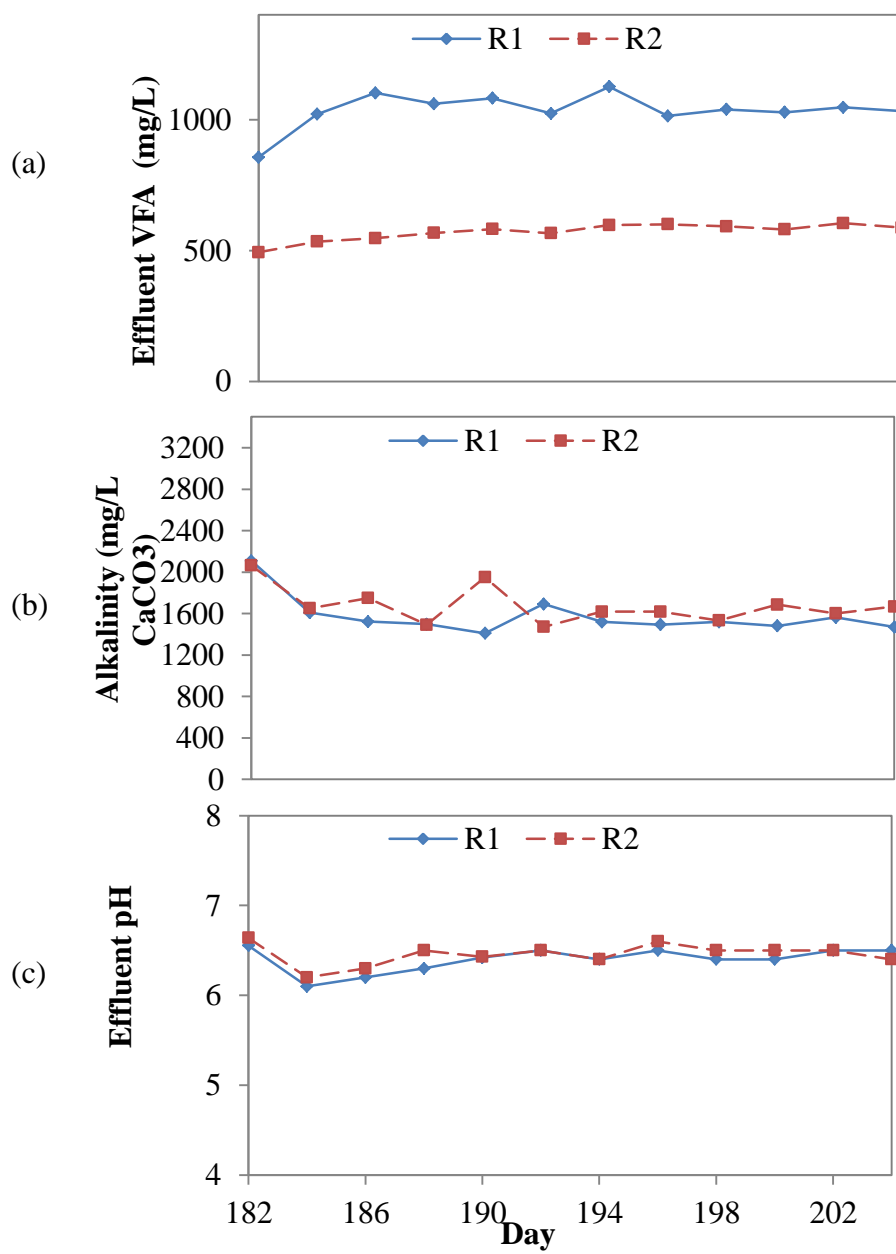


Figure 4.10 Variation of (a) effluent VFA, (b) effluent alkalinity and (c) effluent pH with time at 15 d HRT

4.2.4.3 Effect of LA Treatment and HRT Change– Steady State

Average sulfate reduction of 85% and 58% and average sulfide concentration of 485 mg/L and 277 mg/L were achieved in R1 and R2, respectively in the steady state at 7 d HRT. Methane production was not observed at this stage also in R1 (Appendix E) however, inhibition of MPB resulted in decrease in organic carbon removal efficiency. Only 58 % TOC removal was obtained in the LA treated reactor R1. SRB and MPB as well were affected in R2 by the decrease in the HRT indicated by decrease in sulfate reduction and organic carbon removal. TOC removal of 66% was achieved in R2. VFA built up was observed in both the reactors however the amount of VFA produced in R1 (1030 mg/L \pm 5%) was always higher than in R2 (593 mg/L \pm 5%). The increase in the VFA in R1 in this stage can be attributed to a decrease in sulfidogenic activity shown by an increase in the effluent sulfate concentration. In the control reactor also, increase in VFA can be because of a decrease in SRB activity however, MPB could not use this part of organic carbon indicated by decreased TOC removal efficiency. This phenomenon can be due to the difference in the growth rates of acid producers and MPB. The doubling time of MPB and acid formers is few days and few hours respectively (Bitton, 1994).

4.2.5 Sulfate Reduction and Sulfide Production as a Function of HRT

Overall results are summarized in Table 4.1. Sulfate reduction in both the reactors decreased with the decrease in HRT. In the control reactor (R2), sulfate reduction decreased from 94% to 58% when HRT was decreased from 50 d to 7 d. This reduction was expected because of increase in loading rate and decrease in the contact time between

the biomass and the substrate with the decrease in HRT. Various researchers have reported an improved sulfate reduction at longer HRT. Isa et al. (1986b) reported an increase in sulfate reduction from 65 to 98% when HRT was increased from 0.5 d to 10 d in a high rate reactor with influent sulfate concentration of 1500 mg/L and COD/SO₄²⁻ ratio of 3.33. Neculita et al. (2008) studied the sulfate reduction in downflow column reactor packed with 60% organic matter and 40% inorganic matter. They achieved approximately 40% and 30% sulfate reduction at 10 and 7.3 day HRT, respectively. The influent sulfate concentration in their study ranged between 3440 and 4600 mg/L. Chang et al. (2000) obtained 75% of sulfate reduction at 20 d HRT in a column reactor packed with dried organic matter. The influent sulfate concentration in their study was 2850 mg/L. The lower sulfate removal efficiencies in the studies done by Neculita et al (2008) and Chang et al. (2000) could be because many reasons such as lower operating temperature of 25°C, different reactor configuration and different substrates used. Comparable sulfate reduction of 50-75% at 6-7 d HRT is reported by Nicholas et al. (2005) in a packed bed reactor. The influent sulfate concentration in their study ranged from 1920 to 2400 mg/L.

As mentioned earlier in this section sulfate reduction decreased with a decrease in HRT in the LA treated reactor also. It decreased from 99% to 85% when HRT was decreased from 30 d to 7 d. However, higher amount of sulfate reduction was achieved with the MPB inhibition in the LA treated reactor than the control reactor (Table 4.1). Even though the percent sulfate reduction decreased in both the reactors, the rate of sulfate reduction increased with a decrease in the HRT. It increased from 60 mg SO₄²⁻/L.d to 369 mg SO₄²⁻/L.d in LA treated reactor R1 and from 57 mg SO₄²⁻/L.d to 252 mg SO₄²⁻/L.d in

control reactor R2, as the HRT was decreased from 50 d to 7 d. Even though sulfate was reduced at a higher rate at lower HRT, it was reduced slowly but more efficiently at longer HRT.

Sulfide production at steady state varied between 495-470 mg/L in R1 and from 420 to 270 mg/L in R2 (Table 4.1). The theoretical sulfide produced to sulfate reduced ratio (S^{2-}/SO_4^{2-}) is 0.33. In the present study, this ratio varied from 0.16 to 0.18 in R1 and 0.15 to 0.16 in R2. H_2S gas production was observed throughout the experimental period in both reactors (Appendix E). Various researchers have observed sulfur imbalance in biological sulfate reduction process (Gibert et al., 2004; Greben & Sigama, 2009; Sabumon, 2008). They attributed this imbalance to oxidation of sulfide to sulfur, other intermediate sulfur species formation, and the formation of hydrogen sulfide gas. The sulfur imbalance in the current study can also be due to the similar reasons. Many researchers have tried enhancement of anaerobic sulfate reduction. Comparison of their studies is difficult because of different operating conditions, COD/SO_4^{2-} ratios and reactor configurations. S^{2-}/SO_4^{2-} ratio equal to 0.05 in the study done by Gupta et al. (2007) could be because of H_2S stripping by methane gas production at high COD/SO_4^{2-} ratio 13.8. This ratio being more (0.41) than the theoretical ratio (0.33), in a UASB reactor at COD/SO_4^{2-} of 0.5, is not explained by Omil et al (1997a). Kaksonen et al. obtained S^{2-}/SO_4^{2-} ratio of 0.17 -0.20 in a fluidized bed reactor at COD/SO_4^{2-} of 0.67.

Table 4.1 Summary of results

Phase	R1					R2				
	Start-up	LA Treatment and HRT Study				Start-up	LA Treatment and HRT Study			
HRT	50	50	30	15	7	50	50	30	15	7
pH	7.1	6.9	6.7	6.6	6.5	7.1	7	6.9	6.7	6.6
TS	250 ± 6	482 ± 21	492 ± 8	480 ± 29	473 ± 36	288 ± 14	451 ± 21	420 ± 22	357 ± 15	277 ± 10
Free S ²⁻	148 ± 4	323 ± 14	369 ± 6	379 ± 23	393 ± 29	170 ± 8	284 ± 14	281 ± 15	268 ± 11	219 ± 8
SO ₄ ²⁻ reduction (%)	68 ± 2.5	98 ± 1	99 ± 0.5	95 ± 2	85 ± 1.6	72 ± 1.6	94 ± 2.8	91 ± 2.4	80 ± 2	61 ± 1.9
TOC reduction (%)	96 ± 1	71 ± 1.2	65 ± 3	61 ± 2	58 ± 2	96 ± 2	94 ± 2	82 ± 3	74 ± 2.5	66 ± 2
S ²⁻ /SO ₄ ²⁻	0.12	0.16	0.16	0.17	0.18	0.13	0.16	0.15	0.15	0.15
Rate of SO ₄ ²⁻ reduction (mg/L.d)	41	60	100	193	370	43	57	92	162	252

Free sulfide concentration calculated as per Metcalf et al. (2002)

4.2.6 Competition between SRB and MPB

The steady state data for both UAHR reactors at various HRTs were processed to estimate the fraction of total organic carbon (TOC) removed by methane producing bacteria (TOC_{MPB}) via methanogenic pathways and sulfate reducing bacteria (TOC_{SRB}) via biological sulfate reduction pathway. The results obtained are presented in Figures 4.11 – 4.14. TOC_{SRB} calculations were based on the theoretical amount of organic carbon required for the achieved sulfate reduction and TOC_{MPB} was estimated as a difference between total TOC reduction and TOC_{SRB} . Calculations for the estimated fractions of TOC removed by SRB and MPB are presented in Appendix D.

At HRT of 50 days, comparison of steady state results for control UAHR (R2) (Table 4.1) with the suspended growth master reactors (Appendix B) shows that while TOC removal of 94-96% was maintained, there was a significant improvement in sulfate reduction from 63% to 94%. The estimated fraction of TOC removed by SRB and MPB changed from 56% and 43% in the suspended growth reactors (Appendix B) to 66% and 34% in the control UAHR (Figure 4.11). The corresponding TOC removal by SRB and MPB changed from 523 mg/L and 395 mg/L in suspended growth reactors (Appendix B) to 780 mg/L and 390 mg/L in the control UAHR (Figure 4.11). These results indicate that, the UAHR allowed for the enrichment of SRB whereas MPB activity remained the same. Increase in sulfate reduction in an upflow anaerobic sludge blanket (UASB) reactor as compared to suspended growth reactor is also reported by Erdirencelebi et al. (2007). Both the reactors were fed with glucose as a substrate. Sulfate reduction of 640 mg/L and 940 mg/L was obtained in the suspended growth and the UASB reactor, respectively.

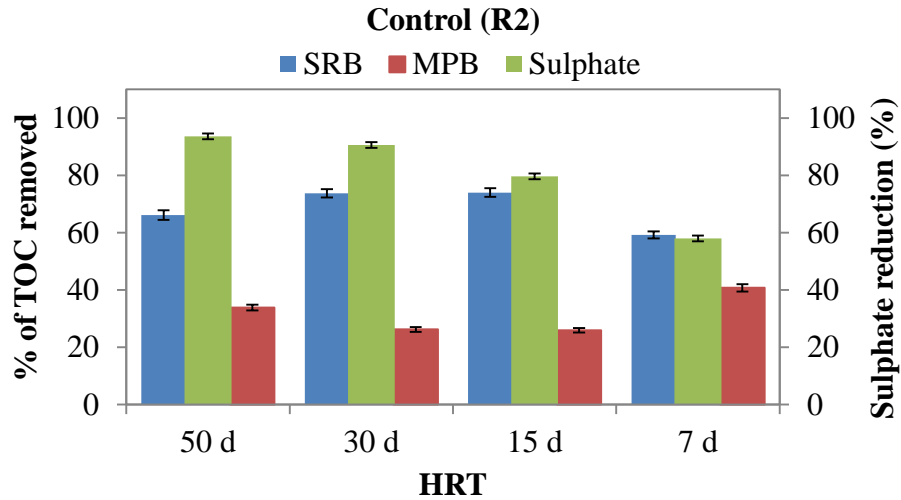


Figure 4.11 Variation of estimated TOC removed by SRB and MPB from total TOC reduction and variation of sulfate reduction with HRT in the control reactor R2.

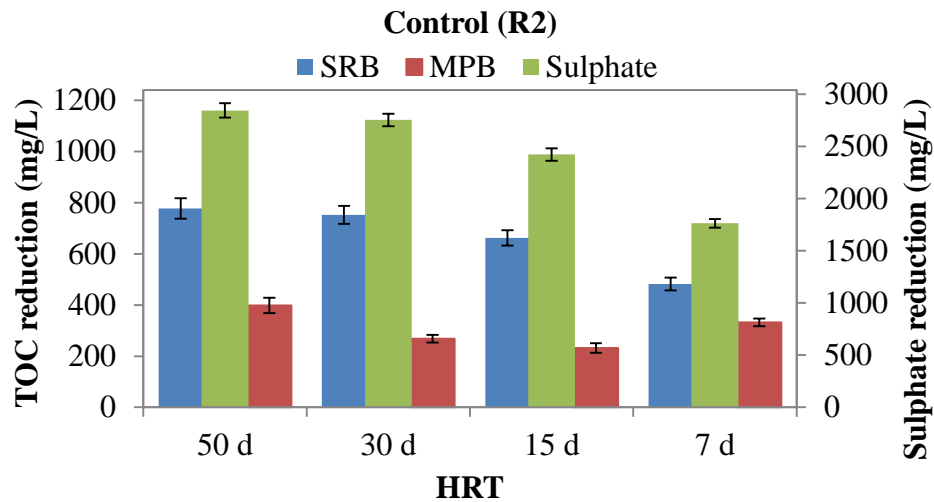


Figure 4.12 Variation of estimated TOC removal (mg/L) by SRB and MPB and variation of sulfate reduction (mg/L) with HRT (d) in the control treated reactor R2

With a reduction in HRT from 50 d to 30 d and 15 d, the fraction of TOC removed by MPB in the control UAHR decreased from 33% to 26% and the corresponding TOC removal by MPB decreased from 400 mg/L to 330 mg/L. During the same period, 66-74% of TOC removed was achieved by SRB (Figure 4.11) giving corresponding TOC removal of 780-660 mg/L (Figure 4.12) in the control UAHR. From these results it can be observed that, sulfate removal by SRB decreased but percent of TOC removed by SRB increased which indicates relative enrichment of SRB as compared to MPB. Free sulfide ($\text{H}_2\text{S}_{(\text{aq})}$) levels during this period ranged between 280 mg/L to 270 mg/L (Table 4.1). The sulfide toxicity and the competition by SRB for the available substrate could be the reasons for lower TOC removal by MPB. Koster et al. (1986) has reported 250 mg/L of free sulfide ($\text{H}_2\text{S}_{(\text{aq})}$) causing 50% inhibition of methanogenesis. With a decrease in HRT from 15 d to 7 d the estimated TOC removal by MPB increased from 26% to 41% and corresponding TOC removal from 230 to 330 gm/L. Significant decrease in the fraction of estimated TOC removed by SRB (74% to 59%) and corresponding decrease in TOC removal by SRB from 660 to 480 mg/L was observed, which indicates more substrate was available for the MPB growth. The free sulfide ($\text{H}_2\text{S}_{(\text{aq})}$) concentration during this stage was 220 mg/L. The reduction of sulfide below the toxic level and more substrate availability could be the reasons for increased TOC removal by MPB. The increase in TOC consumption by MPB also indicates the slow shift of population towards MPB than SRB with a decrease in HRT from 15 d to 7 d.

In the LA treated reactor R1, in the start-up phase at 50 d HRT, equal fraction of TOC was removed by SRB (50%) and MPB (50%) (Appendix D) which changed to 94%

and 6% after addition of LA (Figure 4.13). The corresponding TOC removal by SRB increased from 598 mg/L to 815 mg/L (Appendix D) and the TOC removal by MPB decreased from 592 mg/L to 70 mg/L (Figure 4.14). The increase in TOC can be attributed to the lack of competition from MPB because of inhibition by LA and higher substrate availability. As seen in Figure 4.12 and 4.14, the TOC removal by SRB started declining when HRT was decreased below 50 days in R2 whereas, in R1 this decline was observed after 30 d HRT. The decrease in TOC consumption by SRB with a decrease in HRT indicates an increase in SRB biomass washout at lower HRT and its tendency to loosely adhere to the packing material (Pall rings). However more pronounced effect of SRB biomass washout was seen in the control reactor than the LA treated reactor which could be because of development of more SRB population during 50 d and 30 d HRT period in the LA treated reactor than the control reactor. SRB and MPB population was not enumerated but more TOC removal by SRB than MPB (Figure 4.12 and Figure 4.14) was used as an indication of relative SRB and MPB population. Selective washout of SRB is also reported by Isa et al. (1986a) and Yoda et al. (1997). They attributed this to the better attachment property of MPB than the SRB. Isa et al (1986a) observed 2000 times more MPB population in the biofilm than the effluent whereas SRB population was only 30 times higher in the biofilm than in the effluent. According to the visual observation of El-Bayoumy et al. (1999), SRB did not attach to the packing media but just settled on the packing material and higher SRB population was observed at the bottom of high rate reactor used in their study.

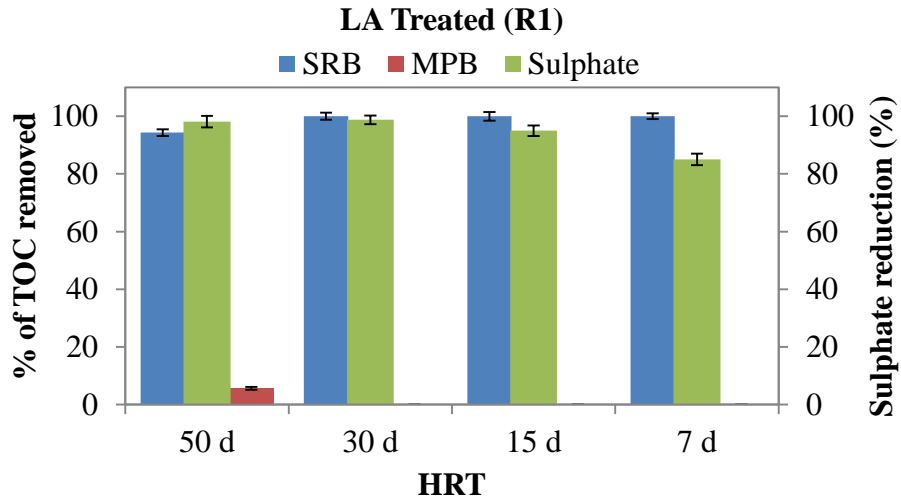


Figure 4.13 Variation of estimated TOC removed by SRB and MPB from total TOC reduction and variation of sulfate reduction with HRT in the LA treated reactor R1

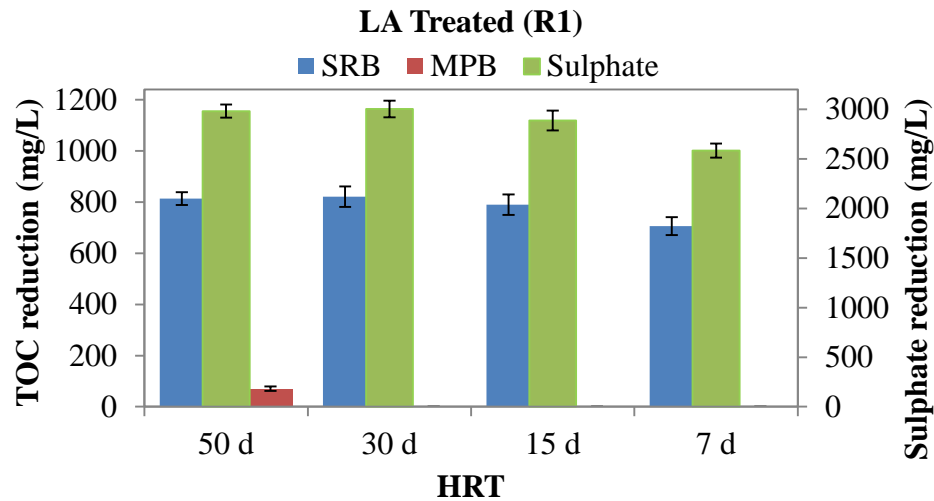


Figure 4.14 Variation of estimated TOC removal (mg/L) by SRB and MPB and variation of sulfate reduction (mg/L) with HRT (d) in the LA treated reactor R1

4.2.7 Effect of LA on Anaerobic Sulfate Reduction in Different Studies

The effect of LA to inhibit MPB and to allow SRB to use the available carbon source more efficiently was studied by Sharma and Biswas (2010) in a batch reactor and Biswas (2012) in a semi-continuous stirred tank reactor (SCSTR). They have reported an increase in the degree of MPB inhibition and improvement in sulfate reduction with an increase in the concentration of LA dose added. In the study done by Sharma et al. (2010), 62%, 66%, 77%, 84%, and 92% of the total sulfate was reduced when 100, 300, 500, 700, and 1000 mg/L LA dose was added, respectively as compared to 24% sulfate reduction when no LA was added. Less than 1% COD diversion towards methane production was reported in cultures receiving 700 mg/L and 1000 mg/L of LA dose, indicating inhibition of MPB. The glucose and sulfate concentrations used in this study were 1870 mg/L COD and 1500 mg/L, respectively. Biswas (2012) used an influent COD concentration of 2333 mg/L and sulfate concentration of 3095 mg/L ($\text{COD}/\text{SO}_4^{2-} = 0.75$) and effect of the varying LA dose was tested in SCSTR. Biswas (2012) obtained 77%, 89%, and 99% sulfate reduction when 250, 750, and 1000 mg/L LA dose was added, respectively. This study was conducted at 40 d HRT. High levels (1400 to 1600 mg/L) of VFA produced in the reactor indicated the inhibition of MPB.

In the present study influent glucose (as COD) and sulfate concentration were maintained at 3040 mg/L. One time dose of LA was added such that 1000 mg/L of LA concentration was achieved in the reactor. Sulfate removal of 99% was obtained at 30 d HRT in a LA treated high rate reactor. Sulfate reduction varied between 99-85% when HRT was changed between 50-7 d. Inhibition of MPB was evident from the absence of

methane gas production in the LA treated reactor (Appendix E). Higher level of sulfate reduction was achieved in the high rate reactor as compared to the SCSTR (Biswas 2012) and the batch reactor (Sharma and Biswas 2010). The similar effects of LA were observed in all the studies mentioned above in terms of MPB inhibition and enhancement of sulfate reduction.

4.3 Metal Precipitation Study- Phase III

As mentioned in Section 3.5, upflow anaerobic hybrid reactors (UAHRs) were operated at 30 d and 15 d HRT again. Metal precipitation by biologically produced sulfide was studied during this phase, which is discussed in Section 4.3.3. This stage corresponds to the second stage of the two stage process described in Section 2.1 (Figure 2.1). The similarities and differences in the steady state performance of the reactors at the same HRT but two different times are discussed in Section 4.3.1.

4.3.1 UAHR Performance at Repeated HRT

The steady state results of repeated 30 d and 15 d HRT stage for the LA treated UAHR R1 and the control UAHR R2 are presented in Table 4.2 and Table 4.3, respectively. The steady state data during the repeated HRT stage is presented in Appendix F. In the LA treated UAHR R1, similar performance in terms of sulfate reduction, sulfide production and TOC reduction was observed as in the previous 30 d HRT stage (Table 4.2). Even after more than 200 days of operation after LA addition,

methane gas production was not observed at repeated 30 d and 15 d HRT also (Appendix E). This is an indication of complete MPB elimination from the system.

In the control UAHR similar performance in terms of TOC reduction was observed but sulfate reduction and sulfide production decreased at the repeated HRT (Table 4.3). Sulfate reduction and sulfide production changed from 91% and 415 mg/L at previous 30 d HRT period to 86% and 357 mg/L at the repeated 30 d HRT (Table 4.3). Similarly, sulfate reduction and sulfide production changed from 80% and 358 mg/L at previous 15 d HRT to 71% and 330 mg/L at the repeated 15 d HRT. The changes in the control UAHR behavior could be attributed to an increase in MPB activity. The estimated fraction of TOC removed by MPB increased from 26% to 35% at 15 d HRT (Table 4.3). In the LA treated reactor consistent performance was observed which could be because of MPB inhibition by linoleic acid. In the system with both SRB and MPB (Control reactor R2), less stability and reproducibility of reactor performance was observed than in the reactor containing only SRB (LA treated reactor R1).

Table 4.2 Performance of R1 at steady state

Reactor	R1			
HRT (day)	30	30	15	15
Duration (day)	105-160	204-264	160-182	264-294
SO ₄ ²⁻ reduction (%)	99 ± 0.5	98 ± 1	95 ± 1	93 ± 1.5
Total sulfide (mg/L)	492 ± 8	488 ± 16	486 ± 29	474 ± 25
Total VFA (mg/L)	725 ± 28	745 ± 30	856 ± 35	885 ± 40
TOC reduction (%)	65 ± 1	63 ± 1.5	61 ± 1.3	57 ± 2.5
TOC _(SRB)	100	100	100	100
TOC _(MPB)	0	0	0	0
pH	6.6	6.6	6.6	6.6

TOC_(SRB)- Estimated fraction of TOC removed by SRB, TOC_(MPB)- Estimated fraction of

TOC removed by MPB (Sample calculation shown in Appendix D)

Table 4.3 Performance of R2 at steady state

Reactor	R2			
HRT (days)	30	30	15	15
Duration (days)	105-160	204-264	160-182	264-294
SO ₄ ²⁻ reduction (%)	91 ± 0.4	86 ± 2	80 ± 2	71 ± 2.5
Total sulfide (mg/L)	415 ± 22	357 ± 30	358 ± 15	330 ± 18
Total VFA (mg/L)	320 ± 15	341 ± 18	493 ± 25	480 ± 20
TOC reduction (%)	82 ± 0.6	81 ± 2.5	72 ± 1.1	74 ± 2
TOC _(SRB)	74 ± 1	71 ± 1.3	74 ± 1.8	64 ± 2
TOC _(MPB)	26 ± 1	29 ± 1.3	26 ± 1.8	36 ± 2
pH	6.7	6.7	6.7	6.7

TOC_(SRB)- Estimated fraction of TOC removed by SRB, TOC_(MPB)- Estimated fraction of

TOC removed by MPB

4.3.2 Metal Precipitation

Simulated acid mine drainage (SAMD) was used to study the precipitation of copper metal. Composition of SAMD is given in Section 3.5. The SAMD pH was adjusted to value 2 by adding concentrated sulfuric acid. Sulfidogenic liquor (SL) and SAMD was mixed in different ratios. The metal precipitation experiments were performed in duplicates and the data is presented in Appendix G. The results are presented in Table 4.4 and 4.5. The pH of the SAMD+SL mixture increased with an increase in the amount of SL added. As seen in Table 4.4, when 70% SL was added, the pH increased from 2 to 5.8. As expected, percentage copper removal increased with an increase in the amount of SL added. The maximum removal of copper (99%-100%) in R1 and (72% -92%) in R2 was

Table 4.4 Copper removal from SAMD using sulfidogenic liquor from R1

HRT (days)	Total sulfide generated in R1 (mg/L)	SAMD/ SL ratio	pH after mixing	Initial copper concentration in mg/L Cu	Final copper concentration in AMD+SL mixture in mg/L Cu	% Cu removal AMD+SL mixture
30	490	3:7	5.8	600	0	100
		5:5	4.6	1000	475	52
		7:3	3.1	1400	952	32
		9:1	2.2	1800	1620	10
15	475	3:7	4.9	600	6	99
		5:5	4.6	1000	514	49
		7:3	2.9	1400	933	33
		9:1	2.2	1800	1630	9

Table 4.5 Copper removal from SAMD using sulfidogenic liquor from R2

HRT (days)	Total sulfide generated in R2 (mg/L)	SAMD/ SL ratio	pH after mixing	Initial copper concentration in mg/L Cu	Final copper concentration in AMD+SL mixture in mg/L Cu	% Cu removal AMD+SL mixture
30	360	3:7	5.7	600	51	92
		5:5	4.6	1000	574	43
		7:3	2.7	1400	1043	26
		9:1	2.2	1800	1656	8
15	330	3:7	4.6	600	167	72
		5:5	4.1	1000	633	37
		7:3	2.7	1400	1061	24
		9:1	2.2	1800	1673	7

achieved at 3:7 SADM/SL ratio. The maximum pH change obtained at this ratio in R1 was from 2 to 4.9 and 2 to 5.8 and in R2 it was from 2 to 4.6 and 2 to 5.7 (Table 4.4 and Table 4.5), at 15 d and 30 d HRT respectively. The higher amount of copper removal from SAMD (Table 4.4 and Table 4.5) using SL from the LA treated reactor R1 than the control reactor R2 was obtained due to higher amount of sulfide produced in R1 (490-475 mg/L) than in R2 (360-330 mg/L).

The ratio of moles of copper removed per mole of sulfide added (Cu/S) was calculated. The sample calculations are shown in Appendix H. Even though percentage copper removal increased with an increase in the amount of SL added, moles of copper removed per mole sulfide added increased with an increase in SAMD/SL ratio. At lower pH more copper was removed per mole of sulfide added as seen in Figure 4.15. Cu/S ratio increased from 1 at pH value 5.8 to 2 at pH value 2. A possible reason for increase in

copper removal per mole sulfide added with a decrease in pH could be reduction of Cu^{2+} to Cu^+ and formation of Cu_2S (chalcocite).

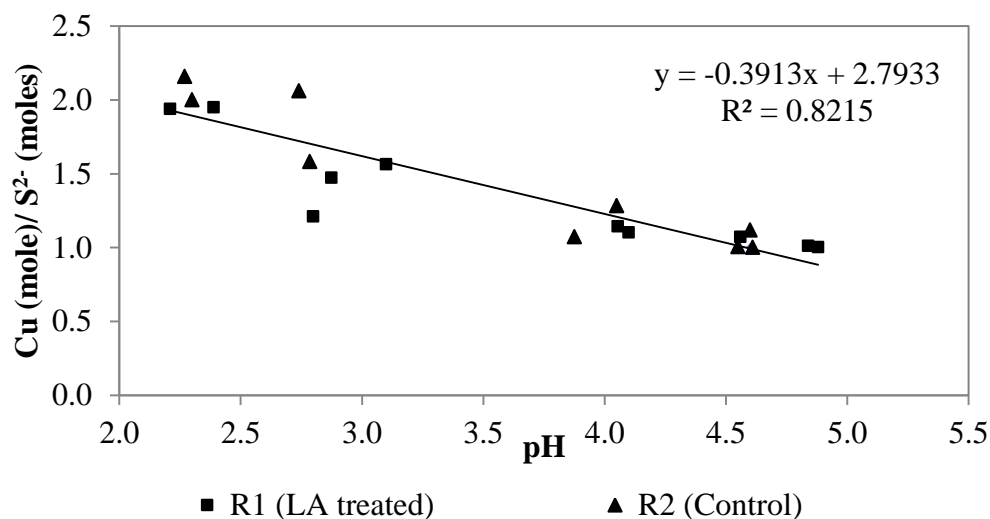


Figure 4.15 Moles of Copper removed per mole S^{2-} as a function of pH

The reactions for CuS (covellite) and Cu_2S (chalcocite) formation are given below.



Reduction of Cu^{2+} to Cu^+ has been previously shown by Weisner et al. (2000). They reported reduction of Cu^{2+} to Cu^+ via sulfur oxidation mechanism during adsorption of copper on the pyrite surface. Patrick et al. has reported formation of several stable, metastable and intermediate copper sulfide complexes (e.g. $\text{Cu}_{1.94-1.97}\text{S}$ djurleite, $\text{Cu}_{1.8}\text{S}$ digenite, $\text{Cu}_{1.75}\text{S}$ anilite, $\text{Cu}_{1.6}\text{S}$ geerite) between CuS and Cu_2S at atmospheric pressure.

Complete neutralization of AMD was not achieved but its pH increased from 2 to 5.8 by the alkalinity generated in SL at 3:7 AMD to SL ratio. Average 99% copper removal was achieved at 3:7 AMD to SL (from LA treated UAHR) ratio. This ratio can be varied depending upon the concentration of metals present in the actual AMD. AMD to SL ratio 6:4 ratio was recommended by Prasad et al. (2009) for 96% of 500 mg/L iron removal from the simulated AMD. The results of the current study indicate the practicability of AMD treatment by biologically produced sulfide. The two stage process described earlier in Section 2.1 can be used successfully used for reducing the acidity and removal of metal content from the AMD.

CHAPTER V

CONCLUSIONS AND RECOMMENDATIONS

4.1 Conclusions

The present study investigated the effect of linoleic acid and hydraulic retention time on anaerobic biological sulfate reduction in semi-continuous upflow anaerobic hybrid reactors (UAHRs) as stage 1 of the two stage process. Based on the studies conducted, the following conclusions can be drawn:

- Biomass retention in UAHR improved sulfate reduction and SRB enrichment as compared to the start-up suspended growth culture. At HRT of 50 days, sulfate reduction in UAHR improved to 98% as compared to 64% in the suspended growth start-up culture. Enrichment in SRB population in UAHR was evidenced by the increase in estimated fraction of TOC reduced diverted to sulfate reduction to 66% as compared to 56% in the start-up culture.
- Linoleic acid can be used to selectively inhibit methane producing bacteria (MPB) while allowing sulfate reduction by sulfate reducing bacteria (SRB) to proceed during biological sulfate reduction in anaerobic high rate reactors. A slug dose of 1000 mg LA/L liquid volume in LA-treated UAHR was sufficient to completely inhibit MPB and ~ 100% of the TOC removed was estimated to be diverted towards sulfate reduction at all HRTs. In the control UAHR, fraction of TOC diverted towards sulfate reduction varied between 74 – 59% for HRTs of 50 – 7 days.

- Sulfate reduction and sulfide production declined with reduction in HRT in both the control and LA treated UAHR. However, higher levels of sulfate reduction and sulfide production were maintained in the LA-treated UAHR as compared to the control UAHR. For HRTs of 50 days to 7 days, in the LA treated UAHR, sulfate reduction > 85%, sulfide levels between 470-500 mg/L were maintained. In contrast, sulfate reduction declined from 94%-58% and sulfide levels from 450-280 mg/L in the control UAHR on reduction over the same range of HRT.

Sulfide produced in stage 1 UAHRs was used for metal precipitation from simulated acid mine drainage (SAMD) containing 2000 mg/L copper and the results showed the following:

- At HRTs of 30 and 15 days, the sulfide produced in LA treated UAHR was sufficient to precipitate 97-100% of copper as compared to 72-92% for the control UAHR.
- Copper precipitated (moles) per mole sulfide increased with a decrease in pH for both the control and LA treated UAHR derived sulfide. The ratio increased from ~ 1.0 at pH 6 to ~2.0 at pH 2.

5.2 Recommendation

1. In a complete sulfidogenic system, if theoretical $\text{COD}/\text{SO}_4^{2-} = 0.67$ is enough for the complete sulfate reduction, could be examined
2. Use of LCFA as an organic carbon source for SRB can be tested.
3. Further research is required in operating the hybrid reactors at HRT lower than 7 days without losing the sulfate reduction efficiency.
4. The possible mechanism of copper precipitation at lower pH could be examined. Cu_2S formation is one of the possibilities. The stability of Cu_2S as compared to CuS and if copper removal at lower pH (2-4) is an attractive alternative as compared to precipitation at higher pH (6-8) can be examined.

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APPENDICES

Appendix A

Description of Semi-continuous Operation

Upflow anaerobic hybrid reactor (UAHR) volume = 8 L

Two UAHRs were operated in a semi-continuous mode. Calculated amount of substrate solution was fed to the reactor and equal amount of reactor content was taken out. The frequency of feeding to the reactor was adjusted such that volume replaced was less than 30% of the UAHR liquid volume.

Sample calculation for 50 day hydraulic retention time (HRT):

50 d HRT :- 8 L liquid volume passes through the reactor in 50 days

Volume to pass through the reactor every day = $8/50 = 0.16$ L/ day

Frequency of feed chosen was 5 days.

Therefore, every fifth day volume to be replaced = $0.16 \times 5 = 0.8$ L = 800 mL

Sample calculation for 7 day HRT:

7 d HRT :- 8 L liquid volume passes through the reactor in 7 days

Volume to pass through the reactor every day = $8/7 = 1.15$ L/ day

Frequency of feed chosen was 2 days so that less than 30% of volume was replaced from the reactor.

Therefore, every second day volume to be replaced = $1.15 \times 2 = 2.3$ L = 2300 mL

Appendix B

Master Reactor Data

Volume of the reactors= 4 L, HRT= 50 days

Temperature = $22 \pm 2^{\circ}\text{C}$

Volume replaced every 10th day= 800 mL

MR1 Data

Day	pH	Sulfate (mg/L)	TOC (mg/L)	Alkalinity (mg/L CaCO ₃)	VFA (mg/L Acetic Acid)
0	6.8	325	1368	1824	851
10	6.8	284	638	1777	851
20	6.6	276	764	1370	1104
30	6.6	255	812	1787	1081
40	7.0	555	772	1934	1081
50	7.2	764	648	2171	817
60	6.8	987	516	2266	473
70	7.1	1066	316	2140	516
80	7.5	1187	387	2013	312
90	7.5	1126	322	1753	235
100	7.3	1202	351	2005	305

Appendix B continued (Master Reactor Data)

MR2 Data

Day	pH	Sulfate (mg/L)	TOC (mg/L)	Alkalinity (mg/L CaCO ₃)	VFA (mg/L Acetic Acid)
0	7.3	415	401	1523	612
10	6.8	383	320	1606	713
20	6.6	449	266	1267	897
30	6.6	407	341	528	1173
40	7.1	358	485	1710	1150
50	7.1	358	440	1506	968
60	7.1	659	419	1459	860
70	7.1	926	373	1504	903
80	7.1	1100	341	1374	602
90	7.0	1199	228	1372	536
100	7.2	1143	246	1507	420

The data presented below is average values of parameters form MR1 and MR2.

Average sulfate removal= 1920 ± 80 mg/L, % sulfate removal = 63 ± 3 %

Average TOC removal= 920 ± 40 mg/L, % TOC removal = 74 ± 4 %

Estimated TOC removal by SRB = 523 ± 25 mg/L

Estimated % of TOC removed by SRB = 56 ± 1 %

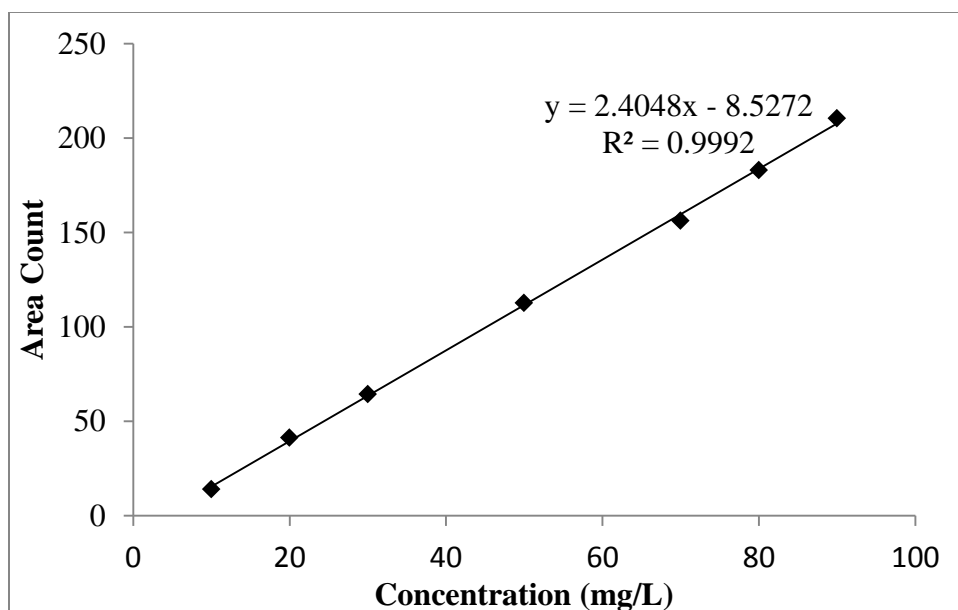
Estimated TOC removal by MPB = 397 ± 40 mg/L

Estimated % of TOC removed by MPB= 43 ± 2.5 %

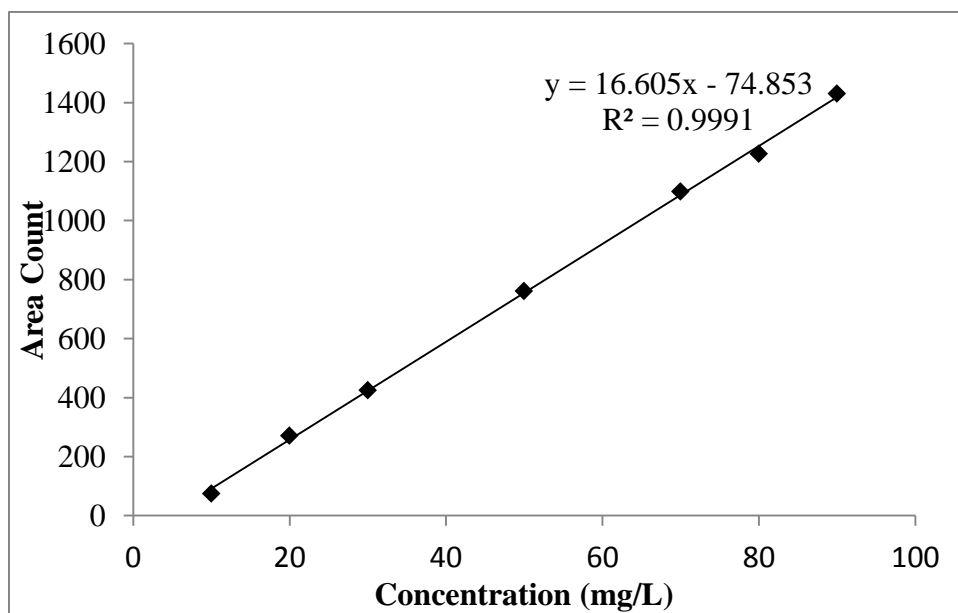
Appendix C

Calibration curves

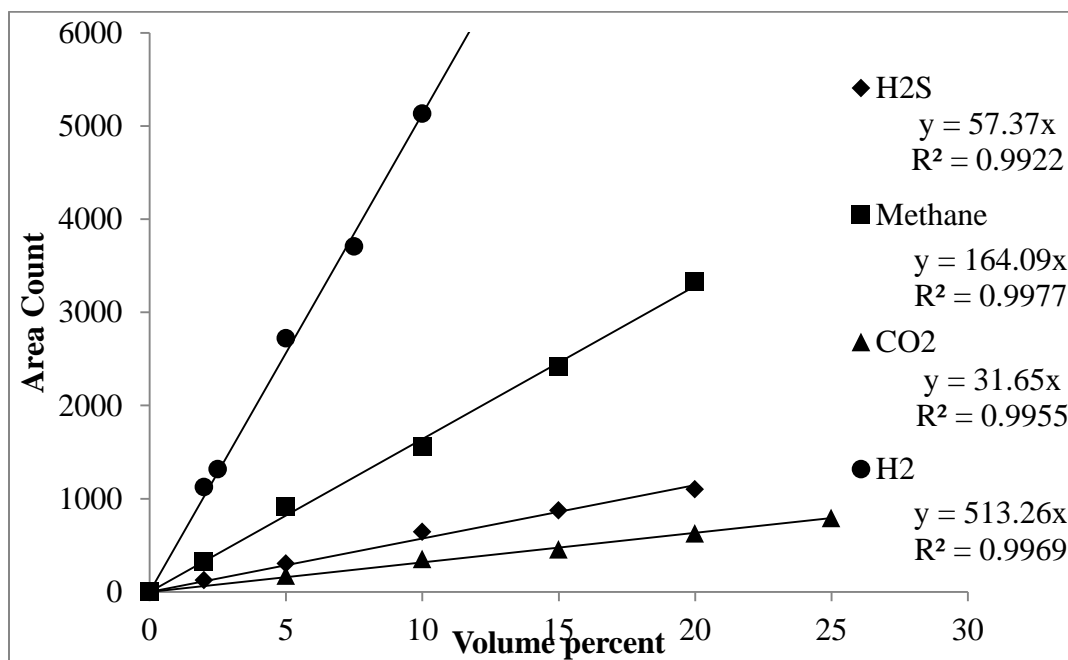
❖ TC calibration curve



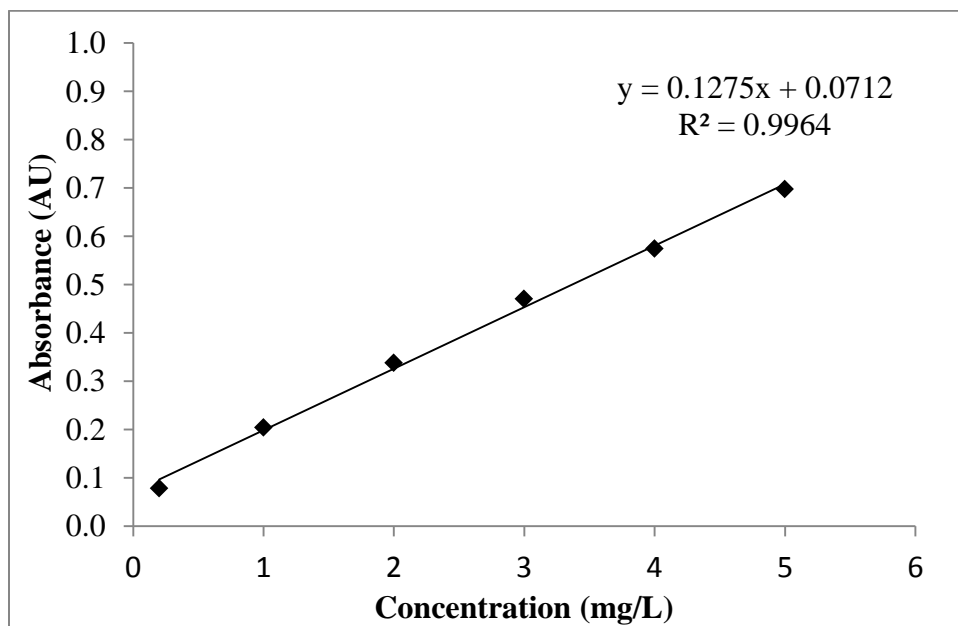
❖ IC calibration curve



❖ Gas calibration curves



❖ Copper calibration curve



Appendix D

Calculation of TOC consumption by SRB and MPB

Assumption: Any TOC removal more than the TOC removed by SRB is accompanied by MPB.

Example: TOC removal by SRB and MPB during the start-up phase in the LA treated reactor R1

Total TOC reduction- 96% (1190 mg/L), SO_4^{2-} reduction- 72% (2188 mg/L),

Influent COD- 3040 mg/L, Influent TOC -1240 mg/L

Theoretically 64 g of COD is required for a 96 g of sulfate reduction

Theoretical $\text{COD}/\text{SO}_4^{2-}$ ratio = $64/96 = 0.67$

Therefore, at $\text{COD}/\text{SO}_4^{2-}$ ratio = 1, 67% COD is consumed for a 100% sulfate reduction.

Since glucose was the only organic carbon source in the feed (Section 3.3), equivalent amount (67%) of TOC is consumed by SRB for 100% sulfate reduction.

For 72% sulfate reduction TOC required = 598 mg/L

TOC by removed by MPB = $1190 - 598 = 592$ mg/L

$\text{TOC}_{(\text{SRB})} = (\text{TOC by SRB} / \text{Total TOC consumed}) * 100 = (598/1190) * 100 = 50.25 \%$

$\text{TOC}_{(\text{MPB})} = (\text{TOC by MPB} / \text{Total TOC consumed}) * 100 = (592/1190) * 100 = 49.75 \%$

Appendix E

Gas Analysis Data

Gas analysis data is presented as a volume % obtained in the headspace.

Hydrogen gas in the headspace throughout the experimental period was less than 1.5%.

HRT (d)	Day	Methane (%)		H2S (%)		CO2 (%)	
		R1	R2	R1	R2	R1	R2
30	105	2	6	13	9	26	28
	115	2	7	12	10	33	36
	125	1	6	16	11	43	42
	135	0	5	17	9	40	46
	145	0	7	14	8	41	38
	155	0	8	17	8	38	42
15	162	0	8	20	8	34	43
	166	0	8	21	7	35	42
	172	0	8	18	8	38	42
	174	0	9	16	8	33	43
	180	0	8	17	7	32	43
7	184	0	7	14	5	38	37
	188	0	8	15	6	38	38
	192	0	11	16	8	35	40
	196	0	10	15	7	33	41
	200	0	9	15	9	35	42
	204	0	10	17	6	33	41
30	214	0	8	15	8	36	41
	232	0	10	13	10	42	43
	250	0	6	16	9	39	38
	262	0	9	15	7	40	36
15	268	0	10	17	8	37	41
	274	0	8	14	6	41	43
	283	0	9	12	9	42	38
	289	0	6	15	7	38	40

Appendix F

Data from Repeated 30 d and 15 d HRT

		TOC (mg/L)		Sulfate (mg/L)		Sulfide (mg/L)		VFA (mg/L)		pH	
HRT	Day	R1	R2	R1	R2	R1	R2	R1	R2	R1	R2
30 d	204										
	214					444	384	681	330	6.6	6.6
	220							762	362	6.6	6.6
	226					485	340	758	342	6.5	6.5
	232	441	236	130	431	461	344	869	416	6.6	6.6
	238			101	477	472	336			6.6	6.6
	244			80	415	482	364			6.6	6.7
	250	454	274	93	439			770	328	6.8	6.7
	256	473	216	72	429	484	358	649	353	6.7	6.7
	262	446	246	101	426	480	349	732	348	6.7	6.7
15 d	265	594	313	281	935	424	315	751	538	6.6	6.6
	268	569	347	265	1059			838	526	6.6	6.6
	271	583	333	288	1006	419	321	866	503	6.5	6.6
	274	572	307	254	885	456	330	751	522	6.2	6.4
	277	544	324	228	821	478	336	1066	473	6.4	6.5
	280			191	918	460	304	953	464	6.5	6.6
	283	492	340	222	935					6.6	6.6
	286	551	312	230	888	479	344	977	506	6.6	6.6
	289			216	880	480	329			6.7	6.7
	292					475	336			6.6	6.6

Blank spaces- parameter not measured

Appendix G

Data from Phase III

Data from the metal precipitation stage (Phase III) for reactor R1 and R2 is presented below.

AMD + SL from LA treated UAHR R1								
HRT	AMD(ml)	SL(ml)	Cu in (mg/L)	Cu out (mg/L)	AMD pH	(AMD+SL) pH	Cu/S ratio	Cu removal (%)
30 d	3	7	600	0	2	5.8	0.9	100
	3	7	600	0	2	5.8	0.9	100
	5	5	1000	450	2	4.7	1.1	55
	5	5	1000	501	2	4.5	1.0	50
	7	3	1400	924	2	3.1	1.6	34
	7	3	1400	980	2	3	1.5	30
	9	1	1800	1638	2	2.2	1.7	9
	9	1	1800	1602	2	2.2	2.1	11
15 d - first run	3	7	600	8	2	5	0.9	99
	3	7	600	15	2	4.8	0.9	96
	5	5	1000	526	2	4.7	1.0	47
	5	5	1000	560	2	4.5	0.9	44
	7	3	1400	896	2	3.1	1.8	36
	7	3	1400	938	2	3	1.6	33
	9	1	1800	1605	2	2.2	2.0	11
	9	1	1800	1688	2	2.2	1.2	6
15 d - second run	3	7	600	0	2	5	0.9	100
	3	7	600	0	2	4.8	0.9	100
	5	5	1000	469	2	4.7	1.1	53
	5	5	1000	500	2	4.5	1.1	50
	7	3	1400	929	2	3.1	1.7	34
	7	3	1400	969	2	3	1.5	31
	9	1	1800	1642	2	2.2	1.7	9
	9	1	1800	1585	2	2.2	2.3	12

Appendix F continued-

AMD + SL from control UAHR R2								
HRT	AMD(ml)	SL(ml)	Cu in (mg/L)	Cu out (mg/L)	AMD pH	(AMD+SL) pH	Cu/S ratio	Cu removal (%)
30 d	3	7	600	60	2	5.7	1.1	90
	3	7	600	42	2	5.5	1.1	93
	5	5	1000	598	2	4.6	1.1	40
	5	5	1000	550	2	4.6	1.3	45
	7	3	1400	1078	2	2.8	1.5	23
	7	3	1400	1008	2	2.6	1.8	28
	9	1	1800	1638	2	2.2	2.3	9
	9	1	1800	1674	2	2.2	1.8	7
15 d - first run	3	7	600	180	2	4.6	0.9	70
	3	7	600	162	2	4.6	1.0	73
	5	5	1000	661	2	4.2	1.0	34
	5	5	1000	620	2	4	1.2	38
	7	3	1400	1036	2	2.7	1.9	26
	7	3	1400	1064	2	2.7	1.7	24
	9	1	1800	1671	2	2.2	2.0	7
	9	1	1800	1656	2	2.2	2.2	8
15 d - second run	3	7	600	144	2	4.6	1.0	76
	3	7	600	180	2	4.6	0.9	70
	5	5	1000	594	2	4.2	1.2	41
	5	5	1000	657	2	4	1.0	34
	7	3	1400	1106	2	2.7	1.5	21
	7	3	1400	1036	2	2.7	1.8	26
	9	1	1800	1656	2	2.2	2.2	8
	9	1	1800	1710	2	2.2	1.3	5

Appendix H

Calculation of Cu/S Ratio

Calculation of moles of copper precipitated per mole sulfide

Example: SAMD- 3 mL, SL- 7 mL, SL sulfide content- 400 mg/L, Initial copper concentration in SAMD- 2000 mg/L, residual copper content in the AMD+SL mixture – 5 mg/L

Initial copper concentration in AMD+SL mixture = $2000 \times 3 / (3+7) = 600$ mg/L

Final Copper concentration = 5 mg/L

Copper removal (%) = $(600-5) \times 100 / 600 = 99.2\%$

Copper reacted = $(600-5) / 63.5 = 9.37$ mole/L

Initial sulfide concentration in SMD+ SL mixture = $400 \times 7 / (3+7) = 280$ mg/L

Initial Sulfide in SMD+ SL mixture = $280 / 32 = 8.75$ mole/L

Moles of copper reacted per mole sulfide = $9.37 / 8.75 = 1.08$ (mole Cu/ mole S)

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